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LARYNGEAL CANCER RECURRENCE

PROGNOSTIC FACTORS AND MANAGEMENT

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ACADEMIC DISSERTATION

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To Anni, Aino and Akseli

If you want to change the world,
pick up your pen and write.
- Martin Luther

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications indicated in the text by their roman numerals:

I Haapaniemi A, Koivunen P, Saarilahti K, Kinnunen I, Laranne J, Aaltonen LM, Närkiö M, Lindholm P, Grénman R, Mäkitie A, Atula T; The Finnish Head and Neck Oncology Working Group. Laryngeal cancer in Finland: A 5-year follow-up study of 366 patients. Head Neck 2016; 38(1): 36-43.

II Haapaniemi A, Väisänen J, Atula T, Alho OP, Mäkitie A, Koivunen P. Predictive factors and treatment outcome of laryngeal carcinoma recurrence. Head Neck 2017; 39(3): 555-563.

III Tiefenböck K*, Haapaniemi A*, Farnebo L, Palmgren B, Tarkkanen J, Farnebo M, Munck-Wikland E, Mäkitie A, Garvin S, Roberg K. Wrap53β, survivin and $p16^{INK4a}$ expression as potential predictors of radiotherapy/chemoradiotherapy response in T2N0-T3N0 glottic laryngeal cancer. Submitted.

IV Haapaniemi A, Kankaanranta L, Saat R, Koivunoro H, Saarilahti K, Mäkitie A, Atula T, Joensuu H. Boron neutron capture therapy in the treatment of recurrent laryngeal cancer. Int J Radiat Oncol Biol Phys 2016; 95(1): 404-10.

* Equal contribution

ABBREVIATIONS

BNCT	Boron neutron capture therapy
BPA-F	Boronophenylalanine-fructose
BSH	Boronosodiumhydrate
CR	Complete response
CRT	Chemoradiotherapy
CT	Computed tomography
DFS	Disease-free survival
DSS	Disease-specific survival
FCR	Finnish Cancer Registry
HPV	Human papilloma virus
IAP	Inhibitor of apoptosis proteins
LC	Local control
LSCC	Laryngeal squamous cell carcinoma
MRI	Magnetic resonance imaging
OS	Overall survival
PET	Positron emission tomography
PR	Partial response
RFS	Recurrence-free survival
RT	Radiotherapy
SCC	Squamous cell carcinoma
TL	Total laryngectomy
TLS	Transoral laser surgery
TNM	Tumor, node, metastasis classification

ABSTRACT

Laryngeal cancer is one of the most common head and neck cancers, with approximately 157000 new cases in the world annually. Laryngeal squamous cell carcinoma (LSCC), the most common histologic type (>90%), mainly affects male smokers. Traditionally, the treatment of early LSCC (T1-2) has been either organ-sparing surgery or radiotherapy (RT). For advanced LSCC (T3-4), surgical treatment has mainly consisted of total laryngectomy (TL), i.e. complete removal of the larynx with resultant permanent tracheostoma and loss of laryngeal voice. To avoid the permanent sequelae of TL, a combination of chemotherapy and RT, chemoradiotherapy (CRT), is being increasingly advocated for the treatment of these tumors. However, it is suspected that this change in treatment paradigm might be behind the observed declining survival of patients with LSCC, a phenomenon noted particularly in the USA. Despite generally good response to the primary treatment, LSCC recurs in up to 30% of cases. Furthermore, recurrence carries a high risk of death due to LSCC. Follow-up is pursued to detect recurrence as early as possible to improve outcome. Although many factors are associated with the risk of LSCC recurrence, no clinically applicable tools exist to aid in primary treatment decisions to avoid recurrence.

In this study, the treatment outcome and recurrences of all patients treated for laryngeal cancer in Finnish university hospitals during 2001-2005 were evaluated. This cohort included 366 patients, which was 67% of all laryngeal cancer patients registered in the Finnish Cancer Registry during the study period. Of the 360 patients with LSCC, 342 were treated with curative intent. Treatment outcomes for glottic T1a LSCC were excellent, with a 5-year disease-specific survival (DSS) of 100%. In the T2 glottic and supraglottic LSCC groups, primarily treated with RT or CRT, DSS was lower than generally reported in the literature (78% and 54%, respectively). CRT was increasingly used for T3-4 tumors, although T4 glottic tumors treated with CRT had poor outcome. Recurrence was observed in 22% of cases; 91% of the recurrences occured within 36 months of treatment. None of the patients with glottic T1a tumors had recurrence after 36 months, which raises the question of the role of a routine 5-year follow-up for this patient group. WHO performance status >0, presence of neck metastases, and non-surgical primary treatment were significant predictors of recurrence. Local recurrence of glottic LSCC could be successfully salvaged. Regional or distant recurrence and any recurrence of supraglottic LSCC carried a poor prognosis. These results underline the importance of sufficiently aggressive primary treatment, particularly for supraglottic LSCC.

Although treatment outcome with RT or CRT is mainly good, some tumors persist or recur after treatment. In these cases, salvage surgery—often TL—is performed. Currently, there are no validated tools to identify patients with radioresistant tumors prior to treatment. Several molecular markers have been investigated in this regard.

Survivin, Wrap53β, and $p16^{INK4a}$ could theoretically be such markers. Survivin is the smallest member of the inhibitor of apoptosis proteins family, which consists of proteins that regulate controlled cell death. Survivin is rarely expressed in normal tissues, whereas its expression is abundant in cancers. It has been associated with increased tumor radioresistance in some studies. On the other hand, others have recognized it as a marker for improved outcome. The Wrap53 gene partially overlaps the p53 tumor suppressor gene. Antisense RNA produced by this gene regulates the function of the p53 gene. Wrap53β, one of the protein products of the Wrap53 gene, participates in DNA double-strand break repair and telomere elongation, which are mechanisms that ensure genomic stability. Disruption of Wrap53β is associated with dyskeratosis congenita, a condition that predisposes to multiple malignant tumors in the head and neck area. Decreased nuclear expression of this protein is associated with poor treatment outcome in many cancers. The expression of $p16^{INK4a}$, a marker used as a surrogate for human papillomavirus infection, is associated with improved outcome in oropharyngeal squamous cell carcinoma. However, a similar link to treatment outcome has not been established for LSCC.

Based on the observed inferior outcome for T2 LSCC in Finland, the aforementioned markers were investigated in a Finnish-Swedish cohort of 149 patients treated with RT or CRT for T2N0M0 or T3N0M0 glottic LSCC to find factors that could predict recurrence. No significant findings regarding survivin were detected, although a trend towards better disease-free survival (DFS) for patients with strong nuclear survivin expression was observed. Regarding Wrap53β, predominantly cytoplasmic expression was associated with poorer DFS and a trend for poorer overall survival was also observed in this group. $P16^{INK4a}$ expression was rare in LSCC patients (7%) and more common among patients under the age of 60. In this younger patient group, none of the patients with $p16^{INK4a}$ expression experienced tumor recurrence. DFS was significantly better in this patient group as well.

Patients with LSCC recurrence after RT or CRT have only one standard option for salvage, which is surgery. While small recurrences may be salvaged with organ-sparing surgery, TL is often the only option. Non-surgical salvage options are being investigated, one of which is boron neutron capture therapy (BNCT). In BNCT, a non-radioactive boron, B_{10}, commonly in a compound of boronophenylalanine-fructose (BPA-F), is infused intravenously. This substance has a tendency to accumulate preferably in tumor cells. After infusion, the tumor is irradiated with epithermal neutrons. This leads to the boron neutron capture reaction, which releases lethal doses of radiation within the cells containing BPA-F. BNCT has the ability to deliver high doses of radiation to the tumor with low toxicity to surrounding tissues.

In the current study, a group of nine patients who were treated with one or two fractions of BNCT for LSCC persisting or recurring after primary RT or CRT was examined. In addition to response and toxicity evaluation, the potential of BNCT as a larynx-sparing salvage treatment was evaluated. Of the eight evaluable tumors, six (75%) responded to BNCT. No serious (Grade 4-5) toxicity was encountered. Despite

good primary responses, only one patient was permanently cured with a preserved larynx. With treatment intensification and dose optimization, BNCT could show potential as a larynx-sparing treatment.

SUMMARY IN FINNISH

Kurkunpääsyöpä on yksi yleisimmistä pään ja kaulan alueen syövistä. Maailmassa todetaan vuosittain noin 157000 uutta kurkunpääsyöpää. Yleisin syöpätyyppi on levyepiteelikarsinooma (>90%), ja siihen sairastuvat ovat useimmiten tupakoivia miehiä. Varhaisen vaiheen (T1-2) syövän hoitona on perinteisesti ollut joko kurkunpäätä säästävä kirurgia tai sädehoito. Myöhäisen vaiheen (T3-4) syövän kirurginen hoito on useimmiten vaatinut kurkunpään poiston. Tässä leikkauksessa muodostetaan pysyvä henkitorviavanne eikä potilas pysty enää tuottamaan luonnollista ääntä. Näiden haittojen välttämiseksi sädehoidon ja solusalpaajahoidon yhdistelmää, kemosädehoitoa, on lisääntyvässä määrin käytetty kurkunpään poiston vaihtoehtona. Hoitolinjan muutoksen on epäilty olevan huononevien hoitotulosten syynä etenkin Yhdysvalloissa. Vaikka kurkunpääsyövän hoitovaste onkin useimmiten hyvä, uusiutuu syöpä jopa 30%:lla potilaista. Uusiutumisen riskitekijöitä on tunnistettu, mutta niiden soveltuvuudesta hoitopäätöksiä ohjaaviksi tekijöiksi ei vielä ole tarpeeksi tutkimustietoa. Uusiutumiseen liittyy suuri syöpäkuoleman riski. Syöpäpotilaita seurataan hoidon jälkeen, jotta uusiutumat havaittaisiin mahdollisimman varhain, jolloin niiden hoito on tuloksellisinta.

Tutkimuksessa selvitettiin vuosina 2001-2005 kaikkien 366:n Suomen yliopistollisissa keskussairaaloissa hoidetun kurkunpääsyöpäpotilaan hoitotulokset ja syövän uusiutumien erityispiirteet. Levyepiteelisyöpä todettiin 360 potilaalla. Näistä 342:lla hoidon tavoite oli parantava. Äänihuulen pienten, T1a syöpien hoitotulokset olivat erinomaiset: yksikään potilaista ei kuollut kurkunpääsyöpään, vaikka tauti uusiutuikin 11%:lla potilaista. T2 luokan syövät hoidettiin pääasiassa sädehoidolla tai kemosädehoidolla. Näiden potilaiden ennuste oli huonompi kuin on yleisesti raportoitu: äänihuulisyöpäpotilaiden 5-vuotiselossaoloennuste oli 78% ja äänihuulen yläpuolista syöpää sairastavien potilaiden 54%. Kemosädehoitoa käytettiin usein kookkaampien T3-4 syöpien hoidossa, mutta T4 luokan äänihuulisyövissä hoitotulokset tällä menetelmällä olivat erittäin huonot. Syövän uusiutuma todettiin 22%:lla potilaista. Uusiutumista 91% havaittiin 36kk sisällä hoidon päättymisestä. Yksikään T1a syöpä ei uusiutunut 36kk seurannan jälkeen ja tämän ryhmän kohdalla rutiininomaisen viiden vuoden seurannan mielekkyys voidaan kyseenalaistaa. Merkittäviä uusiutuman riskitekijöitä olivat WHO:n suorituskykyluokka >0, kaulan etäpesäkkeet alkuvaiheessa ja ei-kirurginen hoito. Paikallisesti uusiutuneen äänihuulisyövän hoitotulokset olivat hyvät. Ennuste oli hyvin huono, mikäli tauti uusiutui muualla kuin paikallisesti tai alkuperäinen kasvain oli ollut äänihuulitason yläpuolella. Nämä tulokset osoittavat, että erityisesti äänihuulitason yläpuolisia syöpiä tulee hoitaa aggressiivisesti.

Vaikka sädehoidon ja kemosädehoidon tulokset ovatkin useimmiten hyvät, osa kasvaimista ei reagoi hoitoon tai uusiutuu myöhemmin. Tällaisissa tapauksissa edetään kirurgiaan, jolloin leikkauksessa useimmiten poistetaan koko kurkunpää.

Toistaiseksi ei ole olemassa keinoja tunnistaa huonosti sädehoidolle reagoivia kasvaimia ennen hoitoa. Monia molekyylaarisia ennustetekijöitä on tutkittu, ja teoriassa tällaisia ennustetekijöitä voisivat olla mm. survivin, Wrap53β ja $p16^{INK4a}$. Survivin kuuluu ohjelmoidun solukuoleman estäjiin ja on tämän ryhmän pienin proteiini. Survivin esiintyy harvoin terveissä soluissa, mutta sen esiintyvyys on runsasta syöpäsoluissa. Survivinia koskevat tutkimustulokset ovat ristiriitaisia: toisissa tutkimuksissa survivin on yhdistetty huonoon sädehoitovasteeseen ja toisissa se näyttäisi ennustavan parempaa hoitotulosta. Wrap53-geeni sijaitsee osin solusykliä keskeisesti säätelevän p53-tuumorisupressorigeenin kanssa päällekkäin, ja siitä p53 geenin lukusuuntaan nähden vastakkaiseen suuntaan tuotettava ns antisense-RNA säätelee p53-geenin toimintaa. Wrap53β, yksi Wrap53-geenin tuottamista proteiineista, osallistuu DNA:n kaksoiskierteen katkosten korjaamiseen ja telomeerien pidentämiseen. Nämä mekanismit tukevat solun geneettistä vakautta. Wrap53β:n toiminnan häiriintyminen on yhdistetty dyskeratosis congenitaan, sairauteen, joka altistaa useille pään ja kaulan alueen syöville. Tämän proteiinin vähäisempi esiintyminen tumassa on yhdistetty huonompaan ennusteeseen useissa syövissä. Papilloomavirustulehduksen sijaismerkkiaineen, $p16^{INK4a}$:n, esiintymisen on todettu liittyvän suunielun syövän parempaan hoitoennusteeseen. Tällaista kytköstä ei ole kuitenkaan havaittu kurkunpääsyövissä.

T2 potilaiden huonon hoitotuloksen vuoksi edellä mainittuja merkkiaineita tutkittiin suomalais-ruotsalaisessa 149 sädehoidetun T2N0M0-T3N0M0 äänihuulisyöpä-potilaan aineistossa uusiutumaa ennustavien tekijöiden löytämiseksi. Survivin ei tuottanut merkitseviä tuloksia, vaikka syövättömän elossaolon ennuste olikin parempi niillä potilailla, joiden kasvainsolujen tumat ilmensivät tätä proteiinia voimakkaasti. Wrap53β:n solulimaan painottuva esiintyminen taas ennusti lyhyempää tautivapaata elossaoloa ja taipumusta lyhyempään elossaoloon ylipäätään. $P16^{INK4a}$ esiintyi harvoin kurkunpääsyövässä (7%:lla). Sen esiintyminen oli yleisempää alle 60-vuotiailla. Tauti ei uusiutunut niillä alle 60-vuotiailla potilailla, joilla proteiini ilmentyi, ja heillä myös syövättömän elossaolon ennuste oli parempi.

Jos kurkunpääsyöpä uusiutuu sädehoidon tai kemosädehoidon jälkeen, ainoana parantavana hoitovaihtoehtona on pidetty kirurgiaa. Pienet uusiutumat voidaan leikata kurkunpäätä säästäen hyvin tuloksin. Useimmiten ainoaksi vaihtoehdoksi jää kuitenkin koko kurkunpään poisto. Tälle toimenpiteelle on etsitty vaihtoehtoja. Yksi tutkimuksen kohteista on boorineutronikaappaushoito (BNCT). BNCT:ssa ei-radioaktiivista booria, yleensä booriyhdisteenä (esim. boorifenyylialaniini-fruktoosi), annetaan potilaalle suonensisäisesti. Tällä yhdisteellä on taipumuksena hakeutua erityisesti kasvainsoluihin. Infuusion jälkeen kasvainta sädetetään neutroneilla. Sädetys johtaa boorineutronikaappausreaktioon, jossa soluihin kerääntynyt boori hajoaa vapauttaen kuolettavan määrän säteilyä. BNCT:n avulla voidaan antaa suuri sädeannos kasvaimeen ja toisaalta samalla minimoida haittavaikutukset ympäröiviin kudoksiin. BNCT:a on tutkittu erityisesti uusiutuneiden, kirurgisen hoidon ulottumattomissa olevien kasvainten hoitomenetelmänä.

Tutkimuksessa annettiin BNCT-hoitoa yhdeksälle potilaalle, joiden kurkunpääsyöpä ei ollut reagoinut sädehoitoon tai kemosädehoitoon tai joiden tauti oli uusiutunut hoidon jälkeen. Potilaat saivat 1-2 BNCT-annosta. Hoitovasteen lisäksi arvioitiin hoidon haittavaikutuksia ja sitä, voisiko BNCT olla elintä säästävä hoitovaihtoehto kurkunpään poistolle. Hoitovaste saatiin arvioitua kahdeksalla potilaalla, joista kuuden (75%) kasvain reagoi BNCT:lle. Vaikka kasvaimet reagoivatkin hoitoon, vain yhden potilaan syöpä parani pysyvästi BNCT:lla ilman kurkunpään poistoa. Vakavia haittavaikutuksia ei havaittu. BNCT saattaisi olla vaihtoehtoinen kurkunpäätä säästävä hoitomuoto, jos hoitoannosta saataisiin tehostettua.

1 INTRODUCTION

Laryngeal cancer is one of the most common head and neck cancers in the world, with an incidence of 157000 new cases each year (1). Tobacco smoking is the main known risk factor; nine out of 10 patients with this cancer type are smokers. Laryngeal cancer mostly affects males, with a male to female ratio of 9:1 (www.cancerregistry.fi). The most common histologic subtype is laryngeal squamous cell carcinoma (LSCC).

LSCC is classified according to its localization into glottic, supraglottic, and subglottic tumors. Glottic tumors comprise the majority of LSCCs (approximately 70%), followed by supraglottic (approximately 25-30%) cancer. Subglottic tumors are rare, comprising approximately 1-2% of LSCCs. The diagnosis of LSCC is based on histological examination of the tumor tissue, usually acquired through laryngomicroscopy under general anesthesia. Clinical examination and radiological imaging are utilized to assess the local extent and metastatic spread of the disease.

The treatment of LSCC depends on the site of presentation and tumor size, as well as the extent of local and metastatic spread. When distant metastases are absent, as in most cases, treatment is generally given with curative intent. Early stage tumors (T1-2) may be treated with either organ-sparing resection (transoral laser surgery [TLS] or open partial resection) or radiotherapy (RT). Locally advanced tumors (T3-4) require either chemoradiotherapy (CRT) or total laryngectomy (TL), i.e. the removal of the larynx and creation of a permanent tracheostoma, followed by postoperative RT. Treatment of the neck metastases in advanced disease is dependent on the primary treatment. When oncological treatment is chosen, the neck is also irradiated (neck dissection and TL reserved for salvage treatment if needed). If TL is performed, neck dissection is performed simultaneously when indicated.

In the recent decades, the use of CRT instead of radical surgery has increased in the treatment of LSCC. Some landmark prospective studies (2,3) have established CRT as a viable alternative for TL with similar survival outcomes. The aim of laryngeal preservation has, however, raised questions in the light of worsening treatment outcomes in some patient groups in USA cancer registry data (4). In Finland, the era of CRT in the treatment of LSCC began in the late 1990s. Detailed national information on treatment results has previously been lacking. According to data from the combined cancer registry of the Nordic countries (NORDCAN) (5), treatment results seem to be improving slowly in contrast to the data from the USA, where outcomes are stable or even worsening in some subclasses (4,6).

There are currently no means to forecast the outcome of primary oncological treatment in LSCC. However, several factors, such as poor general health (7,8), smoking continuation (9), supraglottic tumor localization (10), and higher T class or

stage (11) are associated with increased risk of recurrence. Treatment intensification (e.g. CRT instead of RT) in patients with known high risk of recurrence could prove advantageous and reduce the need for radical surgery later. On the other hand, treatment deintensification in patients with a lower risk of recurrence could potentially diminish the long-term adverse sequelae of treatment. Several molecular factors have been suggested to indicate tumor radioresistance and oncological treatment failure. Among these factors, inhibitor of apoptosis proteins (IAP) has been one of the study targets. Survivin, the smallest member of this family, regulates cell division and apoptosis. High nuclear expression of survivin has been demonstrated to lead to RT failure in *in vitro* studies (12) as well as in clinical patient series. Wrap53β is a coding product of the Wrap53 gene, which partially overlaps the tumor suppressor p53 gene. The functions of Wrap53β include intracellular translocation of different factors (e.g. telomerase and splicing factors) to so-called Cajal bodies, which are sub-organelles found in the nucleus. Cajal bodies manufacture RNA that is required to add nucleotides to the telomere ends by telomerase enzymes. Wrap53β has recently been studied as a potential predictor of RT response. High nuclear expression of Wrap53β has been observed as a predictor of good RT response in head and neck cancer (13). Human papilloma virus (HPV) infection and its surrogate marker, $p16^{INK4a}$ expression, have been identified as predictors of favorable outcome in oropharyngeal carcinoma. HPV/$p16^{INK4a}$ positivity is rare in LSCC (14), and its etiologic role and prognostic potential are not well understood in this cancer type (15).

Despite good primary response to treatment, recurrence of LSCC is common. In the case of recurrence after primary RT/CRT, treatment options are few. Small recurrent tumors can be salvaged with TLS or open partial resection (16). TL is, however, often the only option for salvage. TL results in the loss of laryngeal voice and the introduction of a permanent tracheostoma. Reirradiation has been investigated as a potential curative treatment option in recurrent head and neck SCC. Due to the associated high risk of serious toxicity, it is not widely advocated in treatment protocols. New, less toxic, more targeted forms of RT for recurrence are being investigated. One example of these is boron neutron capture therapy (BNCT), an experimental therapy based on the extremely short-range radiation produced by boron neutron capture reaction. This therapy ideally only harms the cells uptaking boron, thus minimizing the radiation dose to the surrounding non-cancerous tissues (17).

The present study aims to evaluate the site distribution, treatment, and prognosis of LSCC in Finland as well as to assess both the clinical and molecular factors predicting treatment outcome and the risk of recurrence. The effectiveness of recurrent cancer treatment and, specifically, the safety and efficacy of BNCT in the management of recurrent LSCC are also investigated.

2 REVIEW OF THE LITERATURE

2.1 LARYNGEAL CANCER – GENERAL CONSIDERATIONS

2.1.1 EPIDEMIOLOGY AND RISK FACTORS

Laryngeal cancer is the 21st most common cancer worldwide, comprising 1.1% of all new cancers. Men are more prone to laryngeal cancer; 1.9% of all new cancers in men are found in the larynx whereas for women the figure is significantly lower (0.3%). Variability exists between different geographical regions regarding incidence. In developing countries, the age-adjusted incidence in 2012 was 3.5 per 100000, as opposed to 5.1 in more developed countries. (18) In Finland, the incidence of laryngeal cancer has declined over the last decades in men from the 1968-1972 high point of 6.7 per 100000 to 2.1 in 2014. A decrease in females was also observed, from 0.4 to 0.2 (www.cancerregistry.fi).

Smoking (19) and alcohol consumption (20) are widely recognized as the main risk factors for the development of laryngeal cancer. Together, their effect on cancer risk is multiplicative (21). The decline in laryngeal cancer incidence and mortality has followed the declining trend in smoking in many countries (4,22,23).

2.1.2 HISTOLOGY AND CLASSIFICATION

Squamous cell carcinoma (SCC) comprises the vast majority (over 90%) of laryngeal carcinomas (24,25). Other rarely reported histologies include salivary gland type carcinomas (26), e.g. adenoid cystic carcinoma (27), and mucoepidermoid carcinoma (28) as well as non-epithelial cancers including sarcomas (29) and melanoma (30). Lymphoid malignancies also rarely occur in the larynx (24).

LSCC is staged according to the tumor, node, metastasis (TNM) classification updated by UICC and AJCC (Table 1) (31). The larynx is divided into three regions, namely the glottic (the vocal cords), supraglottic (above the vocal cord level), and subglottic regions (starting from 1 cm below the upper surface of the vocal folds). If the tumor grows into multiple regions, the region with the highest tumor volume is defined as the origin. Although lacking an official definition, the term “transglottic” is widely used in clinical medicine to describe tumors extending both above and below the vocal cord level.

Table 1. *Clinical TNM classification of LSCC according to UICC, 7th edition (31)*

T, Primary tumor

TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ

Glottis

T1	T1a: Tumor limited to one vocal cord, normal vocal cord mobility T1b: Tumor involves both vocal cords, normal vocal cord mobility
T2	Tumor extends to supraglottis and/or subglottis, with or without impaired vocal cord mobility
T3	Tumor limited to the larynx with vocal cord fixation and/or invasion of paraglottic space, and/or inner cortex of thyroid cartilage
T4a	Tumor invades through the outer cortex of thyroid cartilage and/or invades tissues beyond the larynx
T4b	Tumor invades prevertebral space, encases carotid artery, or invades mediastinal structures

Supraglottis

T1	Tumor limited to one subsite of supraglottis with normal vocal cord mobility
T2	Tumor invades mucosa of more than one adjacent subsite in supraglottis or glottis or a region outside the supraglottis without fixation of the larynx
T3	Tumor limited to larynx with vocal cord fixation and/or invades any of the following: postcricoid area, preepiglottic space, paraglottic space, and/or inner cortex of thyroid cartilage
T4a	Tumor invades through the thyroid cartilage and/or invades tissues beyond the larynx
T4b	Tumor invades prevertebral space, encases carotid artery or invades mediastinal structures

Subglottis

T1	Tumor limited to the subglottis
T2	Tumor extends to vocal cord(s) with normal or impaired mobility
T3	Tumor limited to larynx with vocal cord fixation
T4a	Tumor invades cricoid or thyroid cartilage and/or invades tissues beyond the larynx
T4b	Tumor invades prevertebral space, encases carotid artery or invades mediastinal structures

N, Regional lymph nodes

NX	Regional lymph node status cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node no greater than 3cm in greatest dimension
N2a	Metastasis in a single ipsilateral lymph node more than 3cm but not more than 6cm in diameter
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension

M, Distant metastasis

M0	No distant metastasis
M1	Distant metastasis

Stage, all subsites

I	T1	N0	M0
II	T2	N0	M0
III	T3	N0	M0
	T1-3	N1	M0
IVa	T4a	N0	M0
	T1-4a	N2a-c	M0
IVb	T4b	N0-2c	M0
	any T	N3	M0
IVc	any T	any N	M1

Distinguished from other malignant tumors, the TNM classification of LSCC includes a functional parameter, i.e. vocal cord mobility to discriminate between T1, T2, and T3 glottic carcinomas and T2 and T3 supraglottic carcinomas. Vocal cord mobility is determined by subjective evaluation and interpretation. Contrary to most other head and neck tumors, the diameter of the tumor in itself is not a defining characteristic for classification. Rather, the involvement of adjacent structures defines the tumor. These properties of LSCC TNM classification have raised debates on the feasibility and reproducibility of this classification for its complexity (32-35). Imaging with computerized tomography (CT) or magnetic resonance imaging (MRI) brings precision to classification, although peritumoral inflammation often complicates the precise assessment of tumor infiltration, particularly the involvement of cartilage (36,37).

2.1.3 CLINICAL PRESENTATION AND DIAGNOSTIC WORKUP

Laryngeal cancer patients typically present with symptoms of hoarseness and sore throat. As the tumor grows, more severe symptoms including dyspnea and dysphagia may follow (38). The presenting symptoms are dependent on the primary tumor localization. Tumors of the glottic region typically present with hoarseness, whereas supraglottic tumors typically present with pressure symptoms such as lump in the throat or throat pain. Due to the early presentation of hoarseness, glottic tumors are generally detected at an earlier stage than supraglottic tumors (24,38,39).

When a patient presents in the outpatient clinic with a suspected laryngeal tumor, a thorough ENT workup including clinical examination as well as fiberoscopic investigation with or without the use of narrow-band imaging (NBI) (40) is conducted. Videolaryngoscopy with stroboscopy may be performed to obtain information on the function and vibrating properties of the affected vocal cord and thus the depth of invasion of the tumor. After clinical examination, an endoscopic evaluation of the tumor and the surrounding hypopharynx and esophagus under general anesthesia is scheduled to obtain biopsies of the tumor and to assess the extent of tumor growth for therapeutic decision-making. The biopsies are examined by a pathologist to confirm cancer diagnosis. Imaging should encompass the larynx and the neck for assessing the extent of the primary tumor. Imaging of the chest (either with conventional x-ray or CT scan) should also be performed to rule out the presence of distant metastases or synchronous primary lung tumors. For T1a glottic tumors, clinical assessment of the primary tumor and the neck and a chest x-ray are usually sufficient for evaluation.

2.1.4 TREATMENT AND PROGNOSIS

Treatment of the primary tumor in LSCC depends on its localization and clinical stage. Generally, monotherapy with surgery or RT is preferred for early T class (T1-2)

tumors. With increasing tumor size, CRT or radical surgery combined with post-operative RT or CRT may be advocated.

TLS was introduced in the late 1980s as a curative treatment option for LSCC. In 1993, Steiner presented his series of 240 patients treated with TLS including neck dissection when indicated (41). The study included a group of 180 patients with glottic Tis-T2 LSCC with normal vocal cord mobility. For this population, local failures were only encountered in 6% of patients and the 5-year OS and disease-specific survival (DSS) were 87% and 100%, respectively. Several authors have later reported similar, excellent outcomes with TLS for early (T1-2) glottic LSCC patients (42-46).

RT is also considered a valid treatment choice for early glottic LSCC. A Dutch group reported a large retrospective institutional series of 1050 T1-2N0 LSCC patients (47). In their series, 5-year local control (LC) and disease-free survival (DFS) and OS were 85% and 81%, respectively. Several authors presented similar oncological results with 5-year DSS rates of 94%-96% for T1 patients and 82-90% for T2 patients (48-52). More recently, RT and CRT were compared in retrospective studies regarding oncological results in the treatment of early glottic carcinomas (particularly in case of T2 tumors), showing improved laryngeal preservation and DFS, but not OS, in the CRT group (53-56).

Several research groups performed retrospective comparisons comparing TLS and RT in T1a patients. Mahler et al. presented a series of 351 patients (57). In their series, RT was administered for T1a patients during 1986-1996 and TLS during 1996-2005. In their study, 5-year DSS for RT and TLS patients was similar (97 vs. 98%), whereas the relative risk for later TL was 12.7 fold in the RT group. Similar findings regarding laryngeal preservation were reported by Schrijvers et al. (58), although patients with deeper infiltrating tumors were excluded from their study. Prospective, randomized studies comparing oncological results are lacking. A meta-analysis by Abdurehim et al. (59) reported no difference in oncological results and voice outcome measures between the studies, although patients treated with TLS had a higher laryngeal preservation rate. A recent Cochrane review (60) revealed only one randomized controlled trial comparing surgery (open partial resection) and RT for the treatment of early LSCC, concluding that evidence for reliable comparisons is lacking. Regarding voice outcomes, only one randomized trial comparing RT with TLS exists (61), presenting slightly less breathiness and a smaller post-treatment glottal gap in patients treated with RT.

The treatment of the primary tumor in early supraglottic LSCC is similar to that of early glottic LSCC. However, neck metastases are very common in early supraglottic carcinoma due to more abundant lymphatic drainage of the area compared to the glottic tumors. Therefore, the management of the neck with either elective neck dissection or RT/CRT is mandatory. In the case of a clearly unilateral primary tumor,

ipsilateral neck treatment may be performed. In the case of centrally or bilaterally located tumors, bilateral neck treatment is recommended (62).

TL has been the gold standard for the management of advanced LSCC (both glottic and supraglottic) since 1873 when Billroth performed the first procedure (63). Despite its oncological virtues, TL results in a permanent tracheostoma and the loss of laryngeal voice, thus permanently affecting the quality of life of the patient (64,65). In the 1990s, an interim report of the Veterans' Affairs (VA) study (2), the first prospective, randomized trial comparing TL to definitive oncological treatment, was published. In their study of 332 patients, induction chemotherapy followed by RT resulted in equal survival compared to TL but with laryngeal preservation. However, local recurrences were more common after non-surgical treatment. The study did not include a formal laryngeal function assessment. Since the VA study, numerous retrospective studies comparing TL and RT/CRT have been published. The majority of them show no difference in survival outcome between treatments (66-70). Some claim surgical treatment to be associated with improved survival for T3 tumors (71,72), while others have found this to be true only for T4 tumors (73,74). According to some studies, surgical treatment is associated with improved locoregional control (66,67,69). However, no additional randomized trials have been presented after the VA study, possibly due to the ethical constraints on conducting such studies in the presence of evidence of comparable survival outcomes with either treatment. Concerns on the feasibility of oncological treatment for advanced laryngeal cancer have been raised due to the declining survival of laryngeal cancer patients in the USA synchronous to the change in treatment protocols (4,75-77)

In Finland, LSCC treatment follows uniform national guidelines set and updated by the Finnish Head and Neck Oncology Working Group. The local head and neck tumor boards are responsible for the implementation of this protocol, and may decide to deviate from it with due cause.

The guidelines for the treatment of LSCC in Finland are as follows:

Glottic carcinoma: T1-2 tumors may be treated with either TLS or RT. CRT is preferred for young T2 patients and patients with large T2 tumors. Treatment of the neck (surgical or oncological) is not necessary for T1-2N0 glottic tumors. For T3 tumors, the primary treatment of choice is CRT (TL as an alternative treatment option). TL is the treatment of choice for T4 tumors. Neck dissection is performed only in case of clinical neck metastasis for T2-3 tumors and electively for all T4 tumors.

Supraglottic carcinoma: T1N0 tumors may be treated with TLS or RT without neck dissection. For T2-4 tumors, CRT is the treatment of choice, with surgery as an alternative option (TLS for T2 tumors, TL for T3-4 tumors). If surgery is chosen, neck dissection is warranted. With CRT, salvage neck dissection is performed only if residual disease is evident after treatment.

Subglottic tumors (rare): Either CRT or TL is used.

For all LSCCs, post-operative oncological treatment follows the same recommendations: RT is administered to all patients with T3-4 tumors treated with surgery and to all patients with a single nodal metastasis. CRT is given if the primary surgical margins are positive, if more than one pathological metastasis in the neck is detected, or if any of the metastatic nodes present extracapsular growth.

Some variability exists between different national guidelines. For example, the Danish DAHANCA guidelines (78) advocate the primary use of definitive non-surgical treatment for all LSCC patients, except for those with extensive tumors with remarkable thyroid cartilage invasion. For T1a glottic LSCC, TLS is also an alternative option to RT. In Sweden, the national guidelines resemble those from Finland, with some exceptions. CRT is not administered to patients with T2 glottic or supraglottic tumors; CRT is not an alternative to TL in supraglottic T4 tumors (79). In 2006, the American Society of Clinical Oncology published their guidelines for the use of larynx-sparing strategies in the management of LSCC (80). The main difference compared to the Finnish recommendation is the preference for open organ-sparing surgery over TLS for T2 glottic and T1-2 supraglottic tumors. In the 21st century, open partial resection has rarely been utilized for the management of LSCC in Finland, possibly due to the risk of morbidity and complications associated with increased aspiration risk. The National Comprehensive Cancer Network (NCCN) 2014 guidelines (81) comply with the Finnish guidelines, with the exceptions of induction chemotherapy followed by RT or surgery as an alternative for T3 and selected T4 tumors (not advocated in Finland), and CRT not being an option for larger T2 tumors.

The general prognosis of LSCC is modest, with a reported 5-year relative survival rate of 60-65% (82-84). However, there seem to be distinct subgroups of LSCC with differing clinical courses and outcomes. Glottic T1 LSCC represents one of these distinct groups with 5-year OS figures commonly exceeding 90%. Curative treatment intent is feasible for the vast majority of LSCC patients, excluding patients with distant metastases and generally patients with extremely extensive primary tumor (T4b) or nodal metastasis (N3).

2.2 LARYNGEAL SQUAMOUS CELL CARCINOMA RECURRENCE

2.2.1 POST-TREATMENT FOLLOW-UP

The aim of head and neck cancer follow-up is to enable the early detection of loco-regional recurrences as well as detection of second primary tumors (85). A recent

recommendation by the European Laryngological Society for the follow-up of LSCC patients expanded this list of aims to include the evaluation of treatment response, monitoring and management of complications, optimization of rehabilitation, promotion of smoking cessation and cessation of excessive alcohol consumption, psychosocial support to patients and their families, and patient counseling and education (86). The society recommends a risk stratification-based follow-up program. Stage I and II tumors are considered low risk tumors and Stage III intermediate risk tumors, both with a recommended total follow-up duration of five years. Stage IV tumors are considered high-risk tumors and warrant a 10-year follow-up according to this recommendation. If the patient presents with a second primary tumor during follow-up, a life-long follow-up is recommended. Regarding the frequency of follow-up, bimonthly follow-up is recommended for the first two years and quarterly to biannual follow-ups for the remaining years. Longer follow-up intervals may be considered for some low-risk tumors (e.g. T1a glottic LSCC treated with TLS with clear margins). Currently, no evidence exists regarding the ideal frequency and length of post-treatment follow-up in LSCC. In Finland, generally, quarterly follow-up visits are recommended for the first post-treatment year, 3-4 month follow-up intervals for the second year, and biannual visits thereafter until five years have passed since treatment completion.

2.2.2 RISK FACTORS FOR RECURRENCE

Several factors have been suggested to predict LSCC recurrence. These factors may be related to diagnostic delay, patient factors, tumor characteristics, and choice of treatment and its implementation.

Teppo et al. investigated the impact of diagnostic delay on LSCC recurrence in Finnish patients (87). They identified professional delay (i.e. the time from the first doctor's appointment at the primary health care to diagnosis of LSCC) of one year or longer as an independent predictor of local and regional failure. Of head and neck cancer patients, LSCC patients are prone to longer diagnostic delays than patients with SCCs of other sites, and these delays seem to have an impact only on LSCC treatment outcome (88).

Brandstorp-Boesen et al. (89) observed an increased risk of recurrence in younger patients (age under 70 years). Of patient-related factors, poor general health has also been identified as a predictor of recurrence (7,8). Additionally, in a series of 117 patients with T1-T2N0 glottic LSCC (90), low pre-treatment hemoglobin reduced 5-year locoregional control after definitive RT. Patient lifestyle may also increase the risk of recurrence. Smoking continuation during RT was identified as an independent predictor of local recurrence in a Dutch study of 549 T1a glottic LSCC patients (9).

Tumor factors play a significant role in the risk of recurrence. In a study by Johansen et al. (10), supraglottic tumors were more prone to recurrence compared to glottic

tumors. This finding was also observed by Brandstorp-Boesen et al. (89). Another study of 5001 patients from Denmark (11) noted an increase in recurrence with increasing tumor T class and stage in glottic LSCC patients. The local extent of the tumor also seems to affect the risk of recurrence; anterior commissure involvement is associated with local failure in some studies (91,92). Nodal metastasis at presentation has also been shown to predict recurrence, particularly later distant metastasis (93-95).

Although conclusive evidence is somewhat lacking, the choice of treatment has also been shown to affect the risk of recurrence in some studies, which have shown an association between non-surgical treatment of Stage III-IV LSCC and increased risk of local recurrence (2,66,67,69). The success of treatment implementation may also affect later tumor control. Treatment gaps reduce the effect of RT and have been shown to predispose patients to recurrence in some studies (96-98). Regarding surgical details, some studies have identified preoperative tracheotomy (99,100) and positive resection margins (99-101) as risk factors for recurrence.

2.2.3 TIME AND PATTERNS OF RECURRENCE

Despite successful primary treatment, LSCC recurrence is fairly common. The reported incidence generally varies around 16-30% (11,102-104). Most of the recurrences occur within the first 2-3 years after treatment (11,105-108). Some new cancer events may occur remarkably later. Lester et al. (109) examined a group of 61 patients with LSCC. They observed that 85% of the recurrences occurred within 1.4 years, and 90% occurred within 3.5 years of treatment completion. However, recurrences were detected as late as 6.6 years into follow-up. The stage of the tumor, whether early or advanced, had no impact on the time to recurrence. Another study including 404 patients treated with TLS for T1a glottic LSCC reported a somewhat longer median time to recurrence (34 months). The latest recurrence in this study was observed 131 months after treatment. The most prevailing mode of recurrence in LSCC is local recurrence (11,107,110,111). A Danish study by Lyhne et al. (11) reported a 30% recurrence rate for glottic LSCC patients treated with RT. Out of the recurrences observed, 93% were local, 11% were regional, and 5% were distant.

2.2.4 TREATMENT AND PROGNOSIS

In case of tumor persistence or recurrence, cure may still be attempted by salvage treatment, i.e. another treatment with a curative aim. For LSCC recurrence after initial surgical monotherapy, RT, CRT, and repeated surgery remain salvage options. With the changes in primary treatment paradigm favoring organ-sparing oncological treatment, an increasing number of patients with recurrent tumors have already undergone RT or CRT, thus making surgical treatment the only standard treatment available. In small recurrent tumors, organ-sparing surgery may be attempted,

including TLS and open partial resections. With more advanced recurrences, TL remains the most reliable standard treatment (112).

For inoperable recurrent tumors, reirradiation may be considered in an attempt for cure, although one should bear in mind the high risk of complications associated with high cumulative radiation doses. Brachytherapy and proton therapy have also been proposed for recurrent head and neck cancer (113,114), although studies on LSCC are lacking. The standard therapy for recurrent, inoperable tumors is palliative chemotherapy.

2.2.4.1 Partial laryngeal surgery

For small, localized recurrences of LSCC, laryngeal preservation surgery is an option in a carefully selected group of patients. Several absolute or relative contraindications for partial laryngectomy have been suggested: arytenoid fixation, interarytenoid invasion, bilateral impairment of vocal cord mobility, contralateral extension of the disease greater than 3mm, preepiglottic extension, remarkable subglottic extension, thyroid or cricoid cartilage invasion, or extralaryngeal spread of the recurrent tumor (112,115). Patient comorbidities, including pulmonary problems, may also hinder the use of conservative surgery as almost all patients undergoing conservation surgery experience at least temporary aspiration after the procedure, exposing them to the threat of severe pulmonary complications.

Ramakrishnan et al. explored the role of TLS in the management of recurrent LSCC in a systematic review and meta-analysis (116). They identified 11 studies with a total of 249 patients who had received TLS for recurrent laryngeal cancer. Primary Tis-T2 tumors formed 92% of the included patients. The pooled LC after repeated TLM was 64%. When taking into account the patients who later underwent salvage TL for recurrence, LC was 88%. The 2-year pooled OS was 75% with a mean larynx-sparing rate of 72%. With such excellent outcomes, TLS remains a viable option for salvage treatment for limited local recurrences.

2.2.4.2 Salvage total laryngectomy

Salvage TL has produced favorable outcome in several studies. In the landmark RTOG 91-11 study of 517 patients (3), Weber et al. (117) examined a subgroup of 129 patients (25%) who underwent salvage TL. TL was performed due to disease progression/recurrence (63%), inadequate treatment response (29%), or functional problems. The majority of the patients (81%) had had a pre-treatment T class of T3 or higher. The overall complication rate was 52-59%, depending on the primary treatment arm. The rate of pharyngocutaenous fistulae was 15-30%. The 2-year OS varied between 69-76%. Van der Putten et al. (118) reported similar results in

patients who had received RT or CRT as their primary treatment for LSCC. The pre-treatment T class was somewhat lower; T1-2 tumors comprised 68% of patients. A pharyngocutaneous fistula rate of 30% and an overall complication rate of 56% were observed. The 5-year OS was 50% and DSS 58%. Comparable outcomes were reported in another study by Li et al. (119). However, the evaluation of these results is difficult, as they were not stratified according to the extent of the recurrent or persistent disease.

2.2.4.3 Reirradiation

Reirradiation with external beam RT has been investigated as a means for organ-sparing curative treatment in recurrent head and neck SCC (120-124). The use of reirradiation has been limited due to the risk of major toxicity and treatment-related morbidity associated with high cumulative radiation doses. Modern RT techniques, such as intensity-modulated radiotherapy (IMRT), may reduce the morbidity associated with reirradiation, but studies on reirradiation in LSCC remain limited (125,126). In a series consisting of 35 patients who underwent IMRT reirradiation with concurrent chemotherapy for recurrent or second primary head and neck cancer, the 1- and 2-year survival rates were 59% and 48%, respectively. The median progression-free survival was 1.7 years (127). However, acute toxicity was high; 91% of the patients had Grade 3 toxicity and 14% had Grade 4 toxicity. Furthermore, four patients died from treatment-related adverse events.

In addition to conventional photon RT, the potential of alternative forms of RT have also been investigated in the treatment of recurrent head and neck cancer. In brachytherapy, the source of ionizing radiation is brought inside the irradiated area via pre-inserted plastic tubes. High doses of radiation may be delivered to the tumor with lower expected toxicity. However, reports on brachytherapy are scarce in head and neck cancer (and anecdotal in LSCC), perhaps due to the inconvenience of catheter placement in this anatomically and functionally challenging area (114,128). Proton therapy is one of the more intriguing new forms of RT. Contrary to photon RT, where maximum energy hits the surface of the target, energy decreasing with depth, in proton radiation, maximum energy may be targeted to the tumor tissue, thus reducing toxicity to the surrounding tissues (129). The results of proton therapy on head and neck SCC recurrence are promising (113).

2.3 BORON NEUTRON CAPTURE THERAPY (BNCT)

One of the more intriguing new methods of RT is boron neutron capture therapy (BNCT). This experimental treatment is based on the preferential uptake of intravenously infused boron into tumor cells and the neutron capture reaction that follows when this tumor is irradiated with epithermal neutrons.

Neutron capture reactions are nuclear reactions in which one or more neutrons collide with an atomic nucleus to form heavier nuclei. In the boron neutron capture reaction ($^{10}B(n,\alpha)^{7}Li$), neutrons collide with ^{10}B to form ^{11}B, which breaks into high linear energy transfer α-particles (^{4}He) and recoiling Lithium-7 nuclei (^{7}Li). A small amount of γ radiation is also produced (Figure 1) (17).

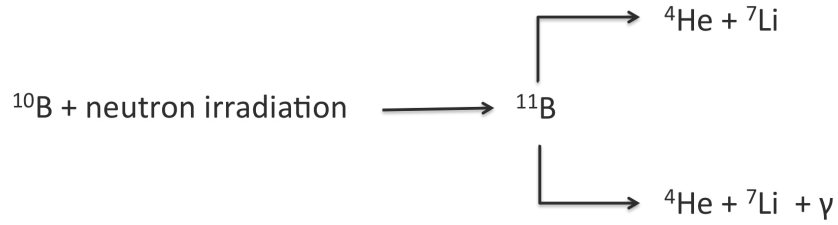

Figure 1 Boron neutron capture reaction

2.3.1 DELIVERY OF BNCT AND CLINICAL APPLICATIONS

From a cancer therapy perspective, the boron neutron capture reaction is appealing. The α-particles and recoiling ^{7}Li nuclei have short path lengths of only 5-9μm in tissue, limiting the lethal radiation effects only to the cells containing ^{10}B. Epithermal neutrons in themselves have very little toxic effect on tissues. Thus, this therapy may also be administered to previously irradiated areas. In addition, hypothetically, weighted doses as high as 60 Gy may be delivered in only one treatment session compared with the 6 to 7 weeks required for the delivery of full-course conventionally fractionated RT (130).

During administration of BNCT, an intravenous infusion of ^{10}B in a carrier substance is first administered prior to neutron irradiation. The prerequisite of BNCT is the preferential uptake of ^{10}B-containing substance by tumor cells. To limit the lethal effects to tumor cells, a requirement of a 3-fold uptake (and a minimum concentration of 20μg/g in tumor) of ^{10}B by tumor tissue compared to normal tissues during the time of neutron irradiation has been accepted. To achieve this, ^{10}B has to be infused in soluble boron compounds. So far, two substances, namely mercaptoundecahydro-*closo*-dodecaborate (or boronosodiumhydrate, BSH) and more frequently (L)-4-dihydroxy-borylphenylalanine-fructose (or boronophenylalanine-fructose, BPA-F) have been used in clinical studies (17). In 2009, Wittig et al. (131) published the results of an EORTC 11001 trial regarding concentrations of BPA-F and BSH in head and neck SCC tissues in patients undergoing surgery after BPA-F or BSH infusion and established the favorable concentration ratio of either substance between tumor tissue and normal tissue. Due to the current lack of means to directly measure ^{10}B levels in tumor tissue during treatment, indirect approximations are made based on ^{10}B concentrations in patient blood samples after BPA-F/BSH infusion and during the neutron irradiation. Some

studies have advocated the potential of positron emission tomography (PET) with radioactively labeled boronophenylalanine (^{18}F-BPA), ^{18}F-BPA-PET in treatment decision-making and dose planning to evaluate the uptake of BPA-F by tumor tissue (132). Thus far, the correlation of ^{18}F-BPA-PET results and tissue ^{10}B concentration has not been established.

After BPA-F/BSH infusion, the tumor area is irradiated with neutrons. In the early days of BNCT, thermal neutrons were used for irradiation. The limitation of thermal neutrons was the inadequate (3-4 cm) tissue penetration, hindering their use in more deeply seated tumors (e.g. of the brain), and necessitating an intraoperative administration during craniotomy. Later, epithermal neutrons with better tissue permeability have been used. The source of the neutron beam has been a fission nuclear reactor, although accelerator-based neutron sources for hospital use are being developed (17).

Currently, the dosimetry and treatment planning of BNCT is limited to estimations relying on indirect methods to assess the ^{10}B concentration in tissues. This has led to different centers administering BNCT to establish their individual dose-calculation methods, which has produced difficulties in inter-study comparability as well as the comparability to photon irradiation (17).

Clinical applications of BNCT have been pursued since the 1950s, when trials on the treatment of glioblastoma patients were conducted in the USA with several different boron-carrier substances (17). Since then, Chadha et al. (133), Kawabata et al. (134), and Kankaanranta et al. (130) conducted studies of BNCT on glioblastoma patients and achieved results similar to conventional therapy. Approximately 100 patients have entered these studies. The use of BNCT for metastatic melanoma has also been reported (135).

BNCT was administered at the Helsinki University Hospital in collaboration with the FiR-1 nuclear reactor in Otaniemi, Espoo until 2012 when the facility was closed. At present, BNCT is administered in Japan, Taiwan and Argentina. The estimated price of BNCT, including all associated costs, was approximately 28000€ per treatment in 2012. Currently, an accelerator-based BNCT facility is being established at Helsinki University Hospital, making this treatment more readily available for patients.

2.3.2 BNCT IN THE TREATMENT OF HEAD AND NECK CANCER

The first study reporting the clinical application of BNCT for head and neck tumor patients was by Kato et al. (136), reporting six patients who had received BNCT for recurrent head and neck cancers (3 SCCs, 2 sarcomas, 1 mucoepidermoid carcinoma). Although initial tumor size reduction was observed in all patients, only one patient had a sustainable complete response (CR). More recently, updates by the same group on their patient cohort have been published (137,138). In the latter report, 62 patients

with unresectable advanced or recurrent head and neck cancer patients were reported. The presented patients had tumors of different head and neck localizations, most commonly oral cavity (39% of patients) and nasal cavity and paranasal sinuses (27%). Histological variability was abundant with 12 different histology types, most commonly SCC (53%) and melanoma (18%). The patients received 1-5 fractions of BNCT. At 6 months post-treatment, 28% of the patients showed CR and 30% partial response (PR). The overall response rates for all patients, patients with newly diagnosed, unresectable tumors, and patients with recurrent tumors were 58%, 39%, and 61%, respectively.

Kankaanranta et al. (139,140) from Helsinki University Hospital conducted the first prospective Phase I/II trial on BNCT in the management of 30 patients with recurrent head and neck cancer. The patients most commonly had tumors of the oral cavity (37%) or nasopharynx (30%), with histologies mainly SCC (80%) or adenoid cystic carcinoma (13%). The majority of the tumors were advanced, T class 3 or 4 (60%) but localized (N0, 73%). BNCT was given in two fractions for 26 patients (87%); the remaining 4 patients received only one fraction. Treatment response at 3 months post-BNCT was reported as CR in 45% and PR in 31% of cases, with an overall response rate of 76%.

Aihara et al. (141) reported their first experiences of 20 patients with advanced primary or recurrent head and neck cancers, which were mostly recurrent SCC (50%) and recurrent non-SCC (35%). The sites of the tumors were not stated. They observed 11 CR and 7 PR responses, amounting to a 90% response rate at 1 month after treatment. The 1- and 2-year loco-regional progression-free survival was 60% and 22%, respectively.

A group from Taiwan reported the results of a Phase I/II prospective study on BNCT for head and neck cancer recurring after RT (142). The patients had previously received a cumulative photon radiation dose of 63-165 Gy. The majority of the patients had tumors of the oral cavity (41%) or nose and paranasal sinuses (24%) with main histologic types being SCC (64%) and other carcinomas (29%). Two patients received only one fraction of BNCT and the other patients received 2 fractions. Of the 14 patients eligible for response assessment 3 months after the last BNCT fraction, 6 patients showed a CR response. The 2-year locoregional control was 28%.

In conclusion, head and neck cancers seem to respond to BNCT. However, the response is often only partial. Unfortunately, response comparisons between different studies are difficult due to various treatment protocols and dose calculation methods, as well as the histological, topographical, and classification heterogeneity of the tumors. No prospective studies comparing BNCT with conventional treatment modalities have been presented.

2.3.3 TOXICITY OF BNCT IN HEAD AND NECK CANCER TREATMENT

Traditionally, conventional photon reirradion is considered to carry an unacceptably high risk of late morbidity (143). BNCT, hypothetically producing a high radiation gradient between tumor tissues and adjacent tissues, has been investigated as a means to deliver lethal doses of radiation to the tumor tissue while minimizing the toxicity to adjacent tissues. Studies on BNCT for head and neck cancer may be divided into those presenting both newly diagnosed and recurrent cancers (136-138,141), and those only presenting cancer recurring after previous photon RT (139,144-146). This heterogeneity makes it somewhat difficult to compare toxicity outcomes between different studies.

Similar to RT toxicity in general, toxicity in BNCT studies is graded according to the Common Terminology Criteria for Adverse Events: 0, no adverse event or within normal limits; 1, mild adverse event; 2, moderate adverse event; 3, severe and undesirable adverse event; 4, life-threatening or disabling adverse event; 5, death related to adverse event. In the study by Suzuki et al. (138) the most common adverse event was transient Grade 3 or 4 hyperamylasemia (27% of patients). Other reported toxicities included mucositis (10%), pain (10%), and fatigue (7%). Three patients (3%) presented with life-threatening carotid hemorrhage, a condition that may also occur irrespective of treatment in some advanced cases. Two of these patients died from carotid rupture. In the study by Aihara et al. (141), no toxicity higher than Grade 2 was reported. In the prospective trials by Kankaanranta et al. (139,140) and Wang et al. (145,146), the most common acute Grade 3 toxicity was mucositis (29% and 53%, respectively). In the former, other common (at least one third of patients) Grade 3 toxicities were also noted: oral pain (53%) and fatigue (33%). The latter reported no other common Grade 3 toxicities. Grade 4 toxicity was reported in only one patient (carotid hemorrhage and laryngeal edema) in the latter trial and in none in the former.

2.4 MARKERS FOR PREDICTING TREATMENT RESPONSE AND PROGNOSIS

2.4.1 GENERAL CONSIDERATIONS

RT is an integral part of LSCC treatment, either in the post-operative setting or as the definitive treatment with curative aim. RT can be administered with or without chemotherapy. Chemotherapy is most often administered concurrently with RT. Curatively aimed oncological treatment carries the potential for organ and function preservation even in the case of selected advanced tumors. Although this organ-sparing approach is generally successful, some patients experience disease persistence after therapy or even disease progression, leading to the need for salvage surgery. In addition, clinically undetectable, radioresistant tumor cell populations

may lead to later recurrence with greater tumor mass (147), thus necessitating TL. If radioresistance could be assessed prior to treatment, these patients could be directed to surgical therapy with the hope of better outcome. Currently, there are no reliable means to assess radioresistance or to predict treatment outcome to guide treatment decisions, although several tools have been investigated.

Epidermal growth factor receptor (EGFR), a member of the tyrosine kinase transmembrane receptor family, is one of the most researched markers for radioresistance and poor prognosis in head and neck SCC. EGFR mediates the actions of epidermal growth factor (EGF), ultimately leading to increased cell proliferation (148). RT induces the autophosphorylation of EGFR, leading to a ligandless activation of the receptor. The resultant increase in cell proliferation (i.e. repopulation) counteracts the tumoricidal effects of RT (149). Overexpression of EGFR is established as a poor prognostic marker in head and neck SCC (150) and in LSCC (151). Cetuximab, a monoclonal antibody targeting EGFR, was investigated with RT by Bonner et al. in a randomized trial on advanced head and neck SCC patients. They found that the addition of cetuximab to RT was associated with improved LC and reduced mortality (152). In their later study, however, no clear improvement in larynx preservation could be observed with the addition of cetuximab to RT (153).

In addition to cancer cell repopulation, cell hypoxia has been identified as one of the main culprits for RT failure. The effect of photon irradiation is delivered to the recipient cells via the formation of free radicals within the cells upon irradiation. These free radicals, short-lived and highly reactive, in turn induce DNA double-strand breaks, which are lethal to the cell. Oxygen interacts with free radicals to prolong their lifetime and thus increases the damage evoked by them. In hypoxic tumor cells, this oxygen interaction is insufficient, which has been shown to increase the radioresistance of these cells three-fold compared to adequately oxygenated cells (154).

Hypoxia elevates the expression of several proteins in the affected cells, including Hypoxia-inducible factor alpha (Hif-1-α), which in turn increases the expression of carbonic anhydrase IX (CA-IX) and glucose transporter type 1 (GLUT-1). Hif-1-α is encoded by the HIF1A gene and regulates adaptive mechanisms to cell hypoxia (i.e. angiogenesis and regulation of cell metabolism and pH), attempting to prevent cell death (154). Schrijvers et al. (155) investigated the predictive value of Hif-1-α, CA-IX, and Glut-1 in a population of 91 patients who had received definitive RT for T1-T2 glottic LSCC. In their study, the expression of Hif-1-α and CA-IX, separately and combined, were identified as independent predictors of local recurrence. Patients with low expression of both Hif-1-α and CA-IX had a significantly lower local recurrence rate than patients who had overexpression of at least one of these markers (6% vs. 32%; p=0.004). Glut-1 did not show predictive value. In a study by Kwon et al. (156), increased expression of Hif-1-α or CA-IX were associated with residual

tumor after RT for T1-T2 glottic LSCC but not with local recurrence. Another study found no predictive role for these factors in supraglottic LSCC (157).

A variety of other potential predictive markers for treatment response have been investigated. Among these are proteins associated with cell damage repair and apoptosis, namely survivin, Wrap53β, and $p16^{INK4a}$.

2.4.2 SURVIVIN

Survivin is the smallest protein in the IAP family. As RT and chemotherapy are believed to trigger apoptosis in cancer cells, IAP poses an interesting target for research on radioresistance. Survivin is expressed in most human tumor cells, whereas its expression is rarely detectable in normal, matured cells. Survivin plays an important role in the regulation of cell division and apoptosis (158). *In vitro* studies on survivin expression and radiosensitivity of tumor cells are contradictory. Some studies reported increased radioresistance with increased expression of survivin in pancreatic cancer cell (159) and LSCC cell lines (12), while other studies have shown an association between radioresistance and survivin downregulation (160). Sun et al. (161) examined the functions of survivin *in vitro* in an oral SCC cell line by knocking out survivin with survivin siRNA. Survivin silencing decreased tumor cell growth and rendered the cells more sensitive to RT. Survivin silencing also enhanced apoptosis.

Engels et al. examined the localization of survivin expression with regard to treatment outcome (162). They concluded that predominantly cytoplasmic survivin (in contrast to nuclear survivin) mediates protection against treatment-induced apoptosis, thus worsening treatment response.

2.4.3 WRAP53β

Wrap53 is a gene located on chromosome 17p13. It partially overlaps the tumor suppressor gene p53. Antisense RNA produced by Wrap53 regulates the functions of the p53 gene. (163) The protein transcripts of the Wrap53 gene include Wrap53α, Wrap53β, and Wrap53γ. Wrap53α stabilizes p53 mRNA, inducing protein p53 in response to DNA damage, thus mediating apoptosis. The functions of Wrap53γ are currently unclear (164).

The Wrap53β protein is the focus of research regarding the Wrap53 gene. Its established functions include trafficking of molecules, such as telomerases to the Cajal bodies, which are sub-organelles associated with RNA production. Wrap53β also has a crucial role in telomere elongation, DNA double-strand break repair, and ribonucleoprotein biogenesis. Disruption of Wrap53β functions is recognized as a key factor in the pathogenesis of dyskeratosis congenita, a condition that predisposes to the development of multiple malignant tumors, and spinal muscular atrophy. The

role of Wrap53β in the development of cancer is two-fold. On one hand, the Wrap53 gene functions as a tumor suppressor gene. On the other hand, Wrap53β has also been shown to possess oncogenic properties (164).

The expression of Wrap53β has been studied in several different cancer types. Diminished nuclear expression of this protein is associated with poor survival in head and neck SCC (13), breast cancer (165), ovarian cancer (166), and colorectal cancer (167). No specific reports exist on Wrap53β expression in LSCC.

2.4.4 P16^{INK4A}

Increasing evidence has been presented on the role of high-risk strains of HPV (especially 16 and 18) on the pathogenesis of head and neck carcinoma (168,169). HPV DNA integration into the genome of mucosal epithelial cells leads to production of oncoproteins E6 and E7, both of which inactivate tumor suppressor p53 and hinder apoptosis. E7 also binds to the Rb protein, disrupting the E2F/Rb complex, leading to the degradation of pRb and eventually removing cell cycle restriction. The release of transcription factor E2F induces the overexpression of p16^{INK4a}. This tumor suppressor protein, p16^{INK4a} (a biomarker for oncoprotein E7 function), has been proposed as a surrogate marker for HPV positivity in tumor tissue with high sensitivity. The specificity of p16^{INK4a} in this respect, however, is lower, (170) pointing to an additional, HPV-independent route for overexpression of p16^{INK4a}. Overexpression of p16^{INK4a} has been demonstrated to predict better treatment responses and survival outcomes in patients with oropharyngeal SCC (168,171,172). Rieckmann et al. (173) investigated the impact of radiotherapy on five HPV-positive and five HPV-negative head and neck SCC cell lines. HPV-positive cells were more radiosensitive and exhibited an increased number of residual DNA double strand breaks compared to HPV-negative cell lines. Cell cycle arrest or increased apoptosis were not observed. Their data suggests that HPV positivity leads to compromised DNA repair capability, resulting in better radiosensitivity.

In non-oropharyngeal head and neck carcinomas, the incidence of p16^{INK4a}/HPV positivity is generally low (14). Varying degrees of p16^{INK4a} positivity have been observed in heterogeneous LSCC patient populations. It has been proposed that HPV may play a role in the pathogenesis of LSCC in non-smokers (174) and in younger patients (175). HPV may also be more prevalent in female patients or patients with regional metastasis (15). However, the role of p16^{INK4a} in predicting treatment outcome and survival in LSCC is controversial, possibly due to the rarity of p16^{INK4a} positivity in LSCC and the heterogeneity of the existing patient series and administered treatments (15,174,176,177).

3 AIMS OF THE STUDY

The general objective of this study was to assess the current status and outcomes of LSCC treatment with curative intent in Finland, focusing on aspects regarding LSCC recurrence, the management of recurrent disease, and prognosis after recurrence. Based on the relatively poor outcome of patients with T2 tumors, a further aim was to explore molecular predictive and prognostic markers for definitive RT/CRT outcome.

The specific aims of the present study were:

1. To assess the treatment outcome of LSCC in Finland during 2001-2005 in the era of definitive CRT.
2. To assess the clinical risk factors for LSCC recurrence as well as prognosis after salvage therapy.
3. To evaluate the predictive and prognostic potential of survivin, Wrap53β, and $p16^{INK4a}$ expression in tumor tissue samples from primary T2N0-T3N0 glottic LSCC patients receiving primary oncological treatment.
4. To evaluate the safety and efficacy of BNCT in the management of recurrent LSCC.

4 PATIENTS AND METHODS

4.1 PATIENTS AND STUDY DESIGN

4.1.1 STUDY I AND II

For Studies I and II, a retrospective patient cohort treated for laryngeal cancer at the five Finnish university hospitals during 2001-2005 was gathered. This patient cohort comprised of 366 patients who were diagnosed with a new primary laryngeal cancer during 2001-2005 and who received their primary treatment (including primary salvage) at these hospitals. In comparison with the Finnish Cancer Registry (FCR) data, this study included 67% of all laryngeal cancer patients registered during the study time. When excluding post-mortem diagnoses and patients without histological or cytological confirmation of cancer from the FCR data, the coverage increased to 73%. The current study and FCR cohorts were similar regarding patient age (median 64 and 65 years, respectively) and the proportion of male patients (93% and 91%, respectively). Advanced-stage tumors were overrepresented in the current study (46%) compared to the FCR data (29%).

Data were gathered on patient and tumor characteristics as well as treatment and follow-up data, including follow-up data from other hospitals if it took place outside the university hospitals. Causes of death were provided by Statistics Finland.

The aim of Study I was to assess laryngeal cancer treatment outcomes in Finland after entering the era of definitive CRT as a treatment option for advanced LSCC. Due to the different prognostic characteristics, tumors with histologies other than SCC as well as patients treated with palliative intent were excluded. This left 342 patients for the final analyses. Although this approach hindered the assessment of the general prognosis of all LSCC patients, this approach was chosen to gain information on the efficacy of curatively intended treatment and to also enable better comparability with results of international patient series (these series usually report only the outcome of patients undergoing curatively intended treatment).

The aim of Study II was to assess factors associated with LSCC recurrence as well as the patterns of recurrence and prognosis after LSCC recurrence diagnosis. Patients with successful primary treatment were taken under evaluation. Thus, in addition to the exclusions in Study I, patients who died of any cause within 3 months of treatment as well as those who had uncontrolled disease progression during treatment were excluded (n=26), leaving 316 patients for the final analyses.

4.1.2 STUDY III

The aim of Study III was to investigate the value of Survivin, Wrap53β, and $p16^{INK4a}$ immunohistochemical staining in predicting oncological treatment outcome and prognosis in LSCC patients.

To minimize the bias associated with material heterogeneity, only glottic LSCC patients with no evidence of metastases (TNM classes T2N0 and T3N0) who had received definitive non-surgical primary treatment (RT or CRT) were investigated. Nordic collaboration with Linköping University Hospital, Linköping, Sweden and Karolinska Hospital, Stockholm, Sweden was pursued; all patients meeting the abovementioned inclusion criteria who were treated at these hospitals during 2000-2009 and for whom biopsy material was available for investigation were included in the study. Clinical data were gathered on patient age and gender, histological grade of the tumor and TNM classification, primary treatment (RT or CRT) with total radiation doses, the dates of treatment completion, possible recurrences, the date and cause of death, the date of last follow-up, and the latest follow-up status.

4.1.3 IMMUNOHISTOCHEMISTRY

After confirmation of tumor tissue presence in the sample, sample sections were mounted on positively charged slides. Deparaffinization was performed (Aqua dePar, Biocare Medical, USA).

For $p16^{INK4a}$ analysis, the CINtec Histology Kit for detection of $p16^{INK4a}$ with a monoclonal mouse antibody (clone E6H4; Mtm laboratories AG, Germany) was used.

For survivin and Wrap53β, sections were pretreated with 10mM citrate buffer (Diva Decloaker [Biocare Medical] and DakoCytomation epitope retrieval solution, respectively). Thereafter, a peroxidase block was performed. Samples were incubated in a 1:400 dilution of polyclonal anti-survivin (Thermo Fisher Scientific, UK) for survivin and 1:1000 dilution of rabbit polyclonal anti-WRAP53-483 for Wrap53β. Samples were then stained with EnVision System-HRP (DAB) kit (DakoCytomation). Sections were counterstained for 1 minute with Tacha's hematoxylin. Positive controls were prepared from biopsy material obtained from patients with high survivin and high Wrap53β expression levels as determined *in vitro*.

One pathologist and two clinicians scored the samples independently without knowledge of clinical data. After individual scorings, a consensus meeting was held to obtain a consensus score for the cases with scoring disagreements.

$P16^{INK4a}$ was scored for staining intensity in both the nucleus and the cytoplasm as follows: none, weak, moderate, and strong staining. In accordance with common

practice, the absence of staining or weak staining were considered $p16^{INK4a}$ negative and moderate or strong staining $p16^{INK4a}$ positive.

For Wrap53β, the staining intensity and subcellular staining localization were scored separately. Due to heterogenous staining pattern of Wrap53β, only the part with the highest intensity of staining was evaluated and categorized as follows: no staining, weak staining, moderate staining, and strong staining. Absence of staining or weak staining was classified as Wrap53β negative. Moderate or strong staining was classified as Wrap53β positive. The staining localization within the cell was categorized into nuclear staining (predominantly nuclear staining or equal staining in the nucleus and cytoplasm), cytoplasmic staining (stronger staining in the cytoplasm), or no staining.

Survivin samples were scored separately for staining intensity in both the nucleus and cytoplasm. A scoring system identical to that with Wrap53β was used.

4.1.4 STUDY IV

The aim of Study IV was to assess the safety and treatment outcome of BNCT in patients with LSCC persisting or recurring after primary oncological treatment. An emphasis was placed on determining if BNCT could be an organ-sparing alternative to TL.

All patients who had undergone BNCT for recurrent LSCC at the Helsinki University Hospital were retrospectively identified. Data on patient demographics, primary tumor classification, and specifics on primary treatment and toxicity were gathered. Prospectively gathered data on recurrent tumor characteristics and classification, as well as BNCT treatment specifics were analyzed. When possible, response evaluation was performed three months after the first BNCT treatment from CT/MRI scans by a radiologist utilizing the Response Evaluation Criteria In Solid Tumors (RECIST) v1.1, 2009 (178). The Common Terminology Criteria for Adverse Events version 3.0 was used to assess treatment toxicity (0, no adverse event or within normal limits; 1, mild adverse event; 2, moderate adverse event; 3, severe and undesirable adverse event; 4, life-threatening or disabling adverse event; 5, death related to adverse event) (179).

Ten patients with BNCT treatment for recurrent LSCC were identified. One patient who had received cetuximab in addition to BNCT and was participating in an ongoing study was excluded, leaving nine patients for the final evaluation.

4.1.5 STATISTICAL METHODS

For studies I-IV, OS, DSS, DFS, and recurrence-free survival (RFS) were calculated with the non-parametric Kaplan-Meier product limit estimate method. A detailed

account on the method of calculation of each outcome variable is presented in the original publications. Significances of differences between survivals according to examined variables (e.g. treatment) were calculated using the log-rank test. For studies II and III, independence of potential variables predicting survival were tested with Cox multiple regression analysis. Results were considered statistically significant if $p < 0.05$.

4.1.6 ETHICAL CONSIDERATIONS

The Helsinki University Hospital Research Ethics Committee approved all studies in this thesis (Dnro HUS429/E06/03, Dnro 35/13/03/04/2009, and Dnro 60/13/03/02/2013). A research permit was obtained for each study from the Helsinki University Hospital Department of Otorhinolaryngology – Head and Neck Surgery. For Study III, an approval by the Finnish National Supervisory Authority for Welfare and Health was acquired (Dnro THL/264/5.05.00/2015). For Study IV, informed consent was obtained in accordance to the Helsinki University Hospital BNCT study protocol.

5 RESULTS

5.1 TREATMENT AND OUTCOME OF LARYNGEAL CANCER IN FINLAND (STUDY I)

Over the five-year period of 2001-2005, 366 patients were treated for laryngeal cancer at the five Finnish university hospitals. SCC was the dominant histology (n=360, 98%). The clinical characteristics and T and stage classifications of the tumors are presented in Table 2. The presence of neck metastases is shown in Table 3. The majority of LSCCs (n=253, 70%) were of glottic origin, the largest single group being T1 glottic LSCC with 114 patients (32% of the SCC cohort). A clear difference in stage distribution was noted between glottic and supraglottic patients; 69% of the glottic LSCC patients presented with early stage (Stage I-II) tumors, whereas only 23% of the supraglottic LSCC patients had early stage tumors. The same was true for T class distribution; 72% of the glottic LSCC patients and 41% of the supraglottic LSCC patients had T1-2 tumors, respectively.

Table 2. *Patient characteristics in Study I.*

Gender, n (%)			Male	338 (92)
			Female	28 (8)
Age			Mean 65 years (median, 64; range, 31-89)	
Histology, n (%)				
SCC	352	(96)		
SCC variant	8	(2)		
non-SCC	6	(2)		

Stage and TNM of SCC tumors (n=360), n (%)

	Glottic	Supraglottic	Transglottic	Subglottic
I	114 (45)	7 (8)	0 (0)	0 (0)
II	61 (24)	13 (15)	0 (0)	1 (20)
III	44 (17)	26 (29)	4 (31)	3 (60)
IV	34 (14)	43 (48)	9 (69)	1 (20)
T1	114 (45)	10 (11)	0 (0)	0 (0)
T2	65 (26)	26 (29)	0 (0)	1 (20)
T3	49 (19)	30 (34)	3 (23)	3 (60)
T4	25 (10)	23 (26)	10 (77)	1 (20)

Abbreviations: n, number of patients; SCC, squamous cell carcinoma; TNM, tumor, node, metastasis classification

Table 3. *Neck metastases in LSCC patients in Study I.*

	Glottic (n=253)				**Supraglottic** (n=89)				**Subglottic** (n=5)	**Transglottic** (n=13)
	T1 n=114	**T2** n=65	**T3** n=49	**T4** n=25	**T1** n=10	**T2** n=26	**T3** n=30	**T4** n=23	**T2-4** n=5	**T3-T4** n=13
N0 (n=291)	114	62	40	16	7	13	16	9	5	9
N+ (n=69)	0	3	9	9	3	13	14	14	0	4
Metastasis rate, %	0	5	18	36	30	50	47	61	0	31

Abbreviations: N0, no neck metastasis; N+, clinical neck metastasis.

Altogether 342 patients received treatment with curative intent (95%). Treatment varied according to tumor stage and localization and is summarized in Table 4.

Table 4. *Treatment of LSCC with curative intent according to T class in Study I.*

	n (%)	**RT**	**CRT**	**Sx**	**RT/CRT+Sx**
Glottic	**T1a**	33 (36)	0 (0)	52 (57)	7 (8)
	T1b	16 (73)	0 (0)	4 (18)	2 (9)
	T2	48 (77)	4 (7)	3 (5)	7 (11)
	T3	10 (22)	18 (40)	6 (13)	11 (25)
	T4	3 (13)	6 (26)	2 (9)	12 (52)
Supraglottic	**T1**	4 (40)	0 (0)	2 (20)	4 (40)
	T2	6 (25)	6 (25)	1 (4)	11 (46)
	T3	4 (14)	10 (36)	2 (7)	12 (43)
	T4	1 (5)	8 (36)	2 (9)	11 (50)
Transglottic	**T3-4**	0 (0)	2 (20)	1 (10)	7 (70)
Subglottic	**T2-4**	2 (50)	0 (0)	0 (0)	2 (50)

Abbreviations: n, number of patients; RT, radiotherapy; CRT, chemoradiotherapy; Sx, surgery.

The 5-year DFS, DSS, and OS for LSCC patients treated with curative intent were 76%, 80%, and 63%, respectively. The results stratified according to tumor localization and T class are summarized in Table 5. DSS in both T2 glottic and supraglottic tumors was worse than in T3 tumors. As a subgroup, only one of the six patients receiving CRT for glottic T4 LSCC was alive and disease-free at the end of follow-up.

Table 5. *Treatment outcome of LSCC patients according to T class in Study I.*

		5-year survival, %		
		DFS	DSS	OS
Glottic	**T1a**	88	100	92
	T1b	82	95	77
	T2	62	78	59
	T3	57	79	64
	T4	51	53	42
Supraglottic	**T1**	69	68	50
	T2	46	54	33
	T3	63	72	43
	T4	60	59	50
Transglottic	**T3-4**	70	62	40
Subglottic	**T2-4**	0	50	0

Abbreviations: DFS, disease-free survival; DSS, disease-specific survival; OS, overall survival.

5.2 PREDICTORS AND OUTCOME OF RECURRENT LSCC (STUDY II)

For Study II, 316 patients (294 male, 22 female; mean age 64 years, range 32-88 years) who successfully completed their primary LSCC treatment and who were without recurrence at 3 months after the end of treatment were extracted from the patient cohort of Study I. The demographics of this patient population are summarized in Table 6.

LSCC recurred in 68 patients (22%). The median time to recurrence was 9 months (range, 3-59 months). The majority of the recurrences (91%) occurred within 36 months of the primary treatment. None of the patients with T1a glottic LSCC experienced recurrence after 36 months of post-treatment follow-up. In glottic LSCC, 84% of the recurrences were isolated local recurrences, whereas for other sites, (supraglottic and subglottic), this figure was only 30%.

The results of the multivariate analysis regarding factors related to higher risk of recurrence are summarized in Table 7. WHO performance status >0, presence of neck metastasis at diagnosis, and non-surgical primary treatment were significant predictive factors for recurrence in general. In addition, WHO performance status >0 (hazard ratio 2.9) and non-surgical primary treatment (hazard ratio 2.4) were identified as independent predictive factors for local recurrence. Female gender (hazard ratio 5.3) and non-glottic tumor localization (hazard ratio 4.9) were independent factors for regional recurrence. No factor proved predictive for later distant metastasis.

Table 6. *Patient demographics for Study II.*

Characteristic		No. of patients	% of patients
WHO performance status			
	0	72	23
	1	212	67
	2-4	13	4
	Missing	19	6
Localization			
	Glottic	231	73
	Other	85	27
Histological grade			
	I	105	33
	II-III	159	50
	Missing	52	17
T classification			
	T1-2	203	65
	T3-4	113	35
N classification			
	N0	270	85
	N+	46	15
Stage			
	I-II	190	60
	III-IV	126	40
Treatment			
	Sx	73	23
	RT	113	36
	CRT	49	16
	Sx+RT/CRT	81	26

Abbreviations: Sx, surgery; RT, radiotherapy; CRT, chemoradiotherapy.
The WHO performance status grades: 0, Fully active, able to carry on all pre-disease performance without restriction; 1, Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature; 2, Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours; 3, Capable of only limited self-care, confined to bed or chair more than 50% of waking hours; 4, Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

Table 7. *Univariate and multivariate analyses of factors predicting laryngeal squamous cell carcinoma recurrence in Study II.*

	recurrence, %	p, univariate	p, multivariate	HR (95% CI)
Age				
<60	24	n.s.	n.s.	
≥60	20			
Gender				
male	20	0.022	n.s.	
female	41			
WHO				
0	14	0.051	**0.037**	**2.1 (1.0-4.3)**
1-4	25			
Localization				
glottic	21	n.s.	n.s.	
other than glottic	24			
Histological grade				
I	21	n.s.	n.s.	
II-III	23			
T classification				
T1-2	20	n.s.	n.s.	
T3-4	24			
N classification				
N0	20	0.111	**0.018**	**2.7 (1.2-6.2)**
N1-3	30			
Primary treatment				
non-surgical	26	0.040	**0.008**	**2.2 (1.2-3.9)**
includes surgery	17			

Significant p-values (<0.05) are shown in boldface. Abbreviations: N0, no nodal metastasis; N+, nodal metastasis; yrs, years; HR, hazard ratio; CI, confidence interval, n.s., non-significant; WHO, WHO performance status. Values with p>0.1 are marked n.s.

Treatment was given with curative intent to 47 patients (69%) for the first recurrence. Survival after the first recurrence depended on the localization of the primary tumor and the localization of recurrence. Patients with recurrence of non-glottic LSCC had a significantly shorter mean survival than patients with recurrence of glottic LSCC (14 vs. 74 months; $p<0.001$). Patients with regional/distant recurrence(s) had a significantly shorter mean survival compared to patients with only local recurrence(s) (8 vs. 83 months; $p<0.001$). Only 4 out of the 16 patients with subsequent second or third recurrences were disease-free at the end of follow-up.

5.3 SURVIVIN, WRAP53β AND P16^{INK4A} IN PREDICTING LSCC TREATMENT RESPONSE AND RECURRENCE (STUDY III)

Tissue samples and clinical data were available for 149 consecutive patients treated with RT/CRT for glottic T2N0 or T3N0 LSCC during 2000-2009 (Helsinki University Hospital, n=64; Karolinska University Hospital, n=75; Linköping University Hospital, n=10). The median age of the patients was 63 years (range, 26-93 years). Patient demographics and treatment characteristics are shown in Tables 8 and 9.

Table 8. *Patient demographics for Study III (modified from Study III).*

Characteristic		No. of patients	% of patients
Age	<60 years	58	39
	≥60 years	91	61
Gender	Male	143	96
	Female	6	4
Smoking	Ever	128	86
	Never	10	7
	N/A	11	7
Histological grade	I	33	22
	II	82	55
	III	16	11
	N/A	18	12
T class	T2N0	105	71
	T3N0	44	30

Abbreviations: N/A, not available.

Table 9. *Treatment characteristics of patients in Study III (modified from Study III).*

Characteristic			No. of patients	% of patients
Treatment	T2N0	RT	94	90
		CRT	11	10
	T3N0	RT	22	50
		CRT	22	50
RT dose		<60 Gy	1	1
		60-69 Gy	99	66
		≥70 Gy	49	33

Abbreviations: RT, radiotherapy; CRT, chemoradiotherapy

Regarding clinical treatment outcome, RFS, DSS, and OS for T2N0 patients were 59%, 92%, and 64%, respectively. For T3N0 patients, the respective figures were 34%, 71%, and 47%. T3N0 patients treated with CRT had significantly better RFS, DFS, DSS, and OS compared to those who received RT. No significant differences in outcome were observed regarding treatment with RT or CRT in T2N0 patients.

We analyzed the expression of $p16^{INK4a}$, Wrap53β, and survivin in tumor samples. One of the tumor samples with survivin staining was lost during the staining process, leaving a total of 148 samples for examination. All samples stained positive for survivin. The majority of the samples, 95, showed predominantly nuclear staining. In 35 samples, the staining was predominantly cytoplasmic. Equal staining intensity in the cytoplasm and nucleus was observed in 18 samples. No differences in outcome measures were detected between negative, positive cytoplasmic, positive nuclear, and positive both cytoplasmic and nuclear groups. However, when the outcomes in the group with positive nuclear staining were observed, patients with strong nuclear staining had a trend towards better DFS than patients with only moderate or weak staining (p=0.091).

Regarding Wrap53β, positive staining was observed in 90 of the 149 tumors (60%; 75 positive nuclear, 15 positive cytoplasmic). When comparing patients with positive Wrap53β staining according to different subcellular localizations, those with positive cytoplasmic staining had a trend towards worse OS (p=0.056) and significantly worse DFS (p=0.022) compared to those with positive nuclear staining. A trend towards worse OS (p=0.072) and DFS (p=0.064) was also observed when comparing patients with positive cytoplasmic staining to patients with negative staining. Comparisons between the positive-staining group as a whole and the negative-staining group regarding outcome proved insignificant. No differences in DSS or RFS were observed between any of the groups.

$P16^{INK4a}$ positivity was observed in 11 patients (7%). No correlation between $p16^{INK4a}$ expression and outcome was observed for the general cohort. Younger patients (<60 years of age) had a significantly higher incidence of $p16^{INK4a}$ expression than older patients (16% vs. 3%; p=0.017). None of the $p16^{INK4a}$-positive tumors in this younger patient population had recurrence compared with the 36% recurrence rate observed in $p16^{INK4a}$-negative tumors (p=0.041). A significant 5-year DFS advantage (100% vs. 50%; p=0.021) in favor of patients with $p16^{INK4a}$-positive tumors was observed. No significant differences were observed in OS (100% vs. 68%; p=0.083) or DSS (100% vs. 86%; p=0.276).

Combining $p16^{INK4a}$, Wrap53β, and survivin scores gave no additional prognostic value.

5.4 BNCT IN THE TREATMENT OF RECURRENT LSCC (STUDY IV)

Ten patients were treated with BNCT at Helsinki University Hospital. The treatments took place during 2006-2012. Patients were treated for persistent (n=3) or recurrent (n=7) LSCC (Study IV, Table 1). One patient was excluded from the analysis due to participation in an unpublished, prospective study concerning adjuvant therapy with BNCT, leaving nine patients (8 male, 1 female) for analysis. The median age of the included patients was 68 years (mean 66 years; range 51-81 years). All patients had previously received photon irradiation to cumulative doses of 38-72 Gy. Seven patients were considered to be TL candidates and two were considered inoperable. BNCT was administered in 1-2 fractions. When two fractions were delivered, the second fraction was administered 1-2 months after the first fraction. The average weighted gross tumor volume BNCT dose was 22-38 Gy per fraction. The ranges of maximum weighted mucosal dose and maximum weighted skin dose were 5-14 Gy and 5-12 Gy.

All except one patient survived at least 3 months after the first BNCT and were eligible for toxicity and response evaluations. Five patients (63%) had early (<90 days after treatment) Grade 3 toxicity: 3 patients each (38%) had stomatitis, fatigue, and oral pain, and 2 patients (25%) had mucositis. Three patients had late Grade 3 toxicity (38%): two patients had oral pain and one patient each had pharyngeal mucositis, stomatitis, and tumor bleed. No Grade 4 or 5 (life-threatening or lethal) toxicity was observed.

Eight patients were evaluable for treatment response 3 months after the first (or only) fraction of BNCT. Two patients achieved CR and an additional four patients achieved PR, amounting to an overall response rate of 75%. The median time to progression of the tumor within the target volume was 6.6 months (3.9-8.3 months).

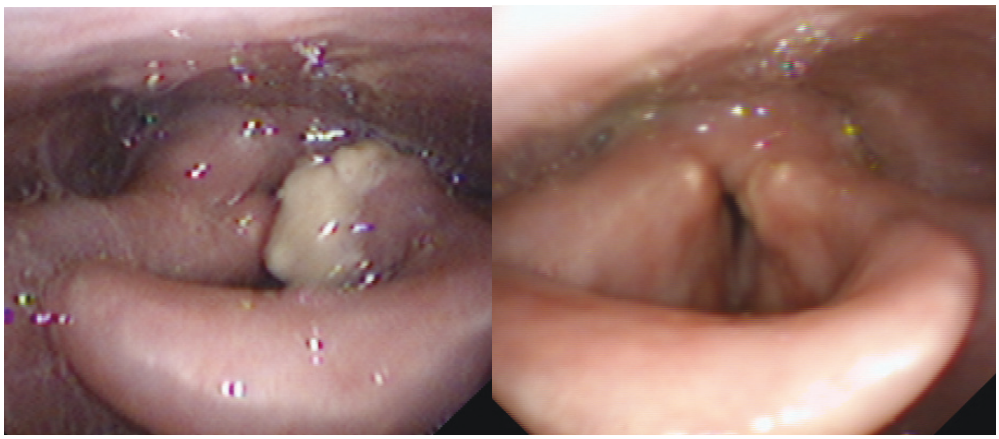

Figure 2 Left: A recurrent supraglottic LSCC detected 18 months after full-dose concomitant chemoradiotherapy. Right: Partial response 4 months after BNCT.

Figure 3 Left: An inoperable LSCC neck metastasis after total laryngectomy, neck dissection and chemoradiotherapy; Right: Partial response 6 months after BNCT.

6 DISCUSSION

Being one of the most studied subsites of head and neck SCC (Pubmed, September 13th 2016, ”laryngeal” AND ”squamous cell carcinoma”, over 9000 references), there are still unanswered questions regarding the ideal choice of treatment, treatment outcome prognostication, and management of recurrences in LSCC. Although often cited as the one of the most common presentations of head and neck SCC, LSCC is divided into clinically distinct subclasses (glottic, supraglottic, subglottic) with different propensity for local spread and metastatic potential, and different treatment approaches and prognosis. Due to the complexity of LSCC classification (a sum of clinical, radiologic, and functional findings), LSCC classification itself poses a potential pitfall for comparisons between different studies that often address a specific group of patients in a retrospective manner, subject to selection bias. An attempt was made in this study to overcome these issues by investigating a national, unselected cohort of LSCC patients treated under uniform guidelines (Study I and II), a selected, consecutive group of LSCC patients with uniform treatment (Study III), and a novel treatment targeted specifically at tumor recurrence after previous RT/CRT (Study IV).

6.1 STUDY STRENGTHS AND LIMITATIONS

For Studies I and II, all consecutive LSCC patients treated at the five University Hospitals in Finland were analyzed. As the treatment of LSCC is centralized in these hospitals and a uniform national treatment protocol is followed at these institutions, the current status of LSCC treatment in Finland could be reliably assessed. However, when comparing the number of patients to those from the FCR, only 67% of the patients registered with LSCC during the study period could be found from the University Hospital registries. Excluding post-mortem diagnoses and unconfirmed histology or cytology from the FCR data increased coverage to 73%. Similar age and gender distributions between FCR and the current data were observed. However, advanced Stage (Stage III-IV) patients were overrepresented in the current data. This confirms the practice of treating a considerable proportion of early stage tumors in non-university hospitals, despite the recommendation for centralizing treatment of LSCC to university hospitals in Finland. However, as the current material includes all patients treated at the University Hospitals following the same Finnish treatment recommendations as other centers, reasonably firm conclusions on the status of current curatively intended LSCC treatment in Finland can reliably be drawn.

In Study II, only patients with initial successful treatment (defined as the patient being alive and without clinical residual disease at 3 months after treatment) were included. Patients with persistent tumors after all treatment and those who had died during or immediately following treatment were excluded. This may have led to the

exclusion of the "worst patients" from the material, thus improving recurrence figures. However, the scope of this study was to concentrate on the events during patient follow-up and the prognostication of recurrence after successful therapy. Therefore, this approach is justifiable.

For Study III, another population of patients and tumor samples was gathered in collaboration with Helsinki University Hospital, Karolinska University Hospital, and Linköping University Hospital. A fairly large material of tumor samples and clinical data (n=149) of uniform tumors (glottic T2-3N0 LSCC) with uniform treatment (RT or CRT) was gathered. This inclusion of such a large, highly specified group of patients with the elimination of some confounding factors related to tumor site, classification, and treatment is scarce in the literature concerning HNSCC prognostication with molecular markers. However, the decision to include only patients with RT or CRT as primary treatment carries a potential for patient selection bias; the patients receiving surgery for glottic T2-3N0 LSCC may have been different from the current material regarding some demographic or tumor-related factors. Nevertheless, this feature of the material did not hinder the pursuit of the main objective of the study, that is, the prognostication of non-surgical treatment outcome in glottic T2-3N0 LSCC. Despite limitations associated with its subjectivity, immunohistochemistry has proven its usefulness in clinical practice for many tumors. However, the assessment of staining intensity is subjective and prone to bias. The heterogeneity of protein expression in tumor material and the variable size of the examined specimens posed another source of potential bias for the assessment of the percentage of tumor cells with protein expression. Having three independent examiners score the samples without knowledge of clinical data minimized this bias.

In Study IV, a small cohort of patients receiving BNCT for recurrent LSCC was examined. Although done in a larger prospective BNCT study setting, the majority of patients in this study were treated as so-called compassionate cases with the associated lack of some follow-up data characteristics (e.g. one patient lacked the proper response evaluation MRI at 3 months after BNCT). Despite this weakness, response assessment could be conducted on all but one patient (one patient died one month after the treatment). The major goal of treatment safety assessment was achieved by clinical record review as well as from the CTCAE forms that had been completed during each visit. Although the number of patients in this study was small, it is the first published study concerning BNCT in a specific locational (larynx) and histologic (SCC) subgroup of head and neck cancer. It is also the first study examining BNCT as a potentially larynx-sparing treatment for radiorecurrent LSCC.

Regarding boron uptake by the tumor, to date no reliable direct standard method exists for its assessment. In the current study, the tumor to normal tissue ratio of 3.5 was assumed based on previous studies (180). This possible inaccuracy of boron concentration assessment made it challenging to evaluate the administered BNCT dose. Therefore, it is difficult to say whether incomplete treatment responses were due to insufficient boron uptake or other factors.

6.2 LSCC IN FINLAND

6.2.1 TREATMENT AND OUTCOME (STUDY I)

Nationwide studies on the treatment of LSCC are rare. In Studies I and II, a nationwide five-year cohort of LSCC patients was examined for subsite and stage distribution, treatment outcome, recurrence, and ultimate survival. In agreement with the current study, the glottis is widely observed as the most common subsite of LSCC (24,181,182), although some have observed supraglottic predominance (183). Mäkitie et al. (22) observed a change in subsite distribution from supraglottic predominance (65%) to glottic predominance (70% in the current series) in Finland over the last decades of the 20th century. The reason for this was speculated to be the simultaneous decline in alcohol and tobacco consumption over those decades. Concurrent with the current findings, subglottic LSCC is recognized as a rare localization, comprising approximately 1-2 % of all LSCCs (24,184-187). Similar to the current study, Bien et al. (24) reported a predominance of T1-2 tumors (54%) in the glottic subsite and predominance of T3-4 tumors (68%) in the supraglottic subsite. This difference in stage distribution between glottic and supraglottic tumors may be explained by variations in symptom profile: glottic tumors lead to hoarseness early on in their course, whereas patients with supraglottic tumors may remain asymptomatic longer.

An excellent treatment outcome for glottic T1 LSCC with either TLS or RT was observed. Similar results with TLS or RT have been presented in numerous studies (47-49,52,57-59,188). Several authors have claimed an increased laryngeal preservation rate in patients treated with TLS compared with RT (57-59). However, these findings come from retrospective series prone to patient selection bias; more deeply infiltrating tumors with greater risk for recurrence may have been treated preferably with RT. Recent systematic reviews regarding the treatment choice for glottic T1 LSCC have found no evidence to support one treatment approach over another (60,189). Aaltonen et al. (61) reported differences in voice breathiness in favor of RT in a randomized trial of 60 patients comparing RT and TLS in the management of T1a glottic LSCC. A meta-analysis by Higgins et al. also noted the superiority of RT over TLS regarding voice outcomes (190). However, in a prospective cohort study of 106 patients (TLS, n=67; RT, n=39), van Gogh et al. (191) noted faster voice recovery in patients treated with TLS regarding jitter, shimmer, and noise energy. They concluded with a recommendation of TLS over RT in the management of T1a glottic LSCC.

One of the advantages of TLS over RT is the shorter time spent in treatment. TLS is ideally a one-session treatment while a full-course RT usually takes 6-7 weeks. Regarding treatment cost (including the hidden cost of missed work hours), TLS appears less expensive (192,193). The vast retrospective evidence shows similar, excellent oncological outcomes with either treatment, yet somewhat conflicting voice

outcome results. Large, prospective trials with adequately long follow-up are needed to find potential differences between treatments regarding long-term adverse effects (including potential second malignancies following RT for LSCC), voice outcome, and quality of life.

In this study, both glottic and supraglottic T2 tumors had remarkably poorer treatment outcome than expected. As early T class tumors, one would expect the outcomes to be closer to T1 tumors and not T3 tumors as was observed in the current study. Most of the glottic T2 patients received RT (77%) or CRT (7%) as their only primary treatment. In the supraglottis, T2 tumors had the worst outcome among all T classes. Half of these patients received RT or CRT as their primary treatment. Unfortunately, due to the relatively small size of these sub-cohorts, comparisons between treatment approaches (non-surgical vs. surgical) could not be conducted. A recent study based on the Surveillance, Epidemiology, and End Results (SEER) database (194) examined the treatment outcomes of Stage I (T1N0) and Stage II (T2N0) glottic LSCC. Significantly poorer DSS for Stage II patients was observed compared to Stage I patients (94% vs. 87%; $p<0.0001$), despite the treatment being identical for both groups. They concluded that Stage II patients might benefit from more aggressive therapy either by combining surgery and post-operative RT or by definitive CRT. Study III showed no survival advantage for T2N0 patients treated with CRT compared to those who were treated with RT alone. However, the number of patients treated with CRT was small. The aforementioned data from SEER also included patients (5%) with no treatment. Among these patients, Stage I patients had a significantly better DSS than Stage II patients (93% vs. 67%; $p<0.0001$), suggesting a difference in tendency for tumor progression. Haugen et al. investigated the feasibility of an accelerated-hyperfractionated RT schedule in the treatment of T2N0 glottic LSCC (195). In their study, T1N0 glottic LSCC patients were given a total dose of 62.4 Gy in once-a-day fractions over 6.5 weeks, whereas T2N0 patients were treated with a split-course hyperfractionated-accelerated RT to a total dose of 64.6 Gy over 4.5 weeks. The oncological outcome for both groups was similar, indicating a potential benefit in shortening the overall treatment time for T2N0 glottic LSCC. The merits of accelerated RT are further supported by Overgaard et al. (196), who investigated the benefit of giving six fractions per week instead of five in a population of 1476 head and neck cancer patients. They observed significantly better LC and DSS in the group receiving six fractions per week. OS, however, did not differ between the groups. Regarding early supraglottic LSCC, Arshad et al. (197) (also based on SEER data) demonstrated superior outcomes with organ preservation surgery compared to RT alone, although this study was prone to selection bias due its retrospective nature.

The treatment outcome of advanced glottic and supraglottic LSCC in the current material with 5-year DSS of 79% and 72% for T3 tumors and 53% and 59% for T4 tumors is well in line with other reports describing treatment results of these tumors using various surgical and oncological treatment approaches (69,198-201). Traditionally, large-volume T4 tumors have been considered unsuitable for definitive oncological treatment (202), concurrent with findings from the current study

regarding T4 glottic LSCC, although some retrospective reports have challenged this principle (203,204). In the early 1990s (2) the concept of tumor response to induction chemotherapy was introduced as a method of treatment selection between radiotherapy and TL in advanced LSCC. With later results indicating an advantage of CRT over induction chemotherapy followed by RT (3), CRT without induction chemotherapy was widely adopted as the mainstay of non-surgical treatment in advanced LSCC. A recent study by Wolf et al. (205) reported the treatment outcomes of an unselected patient series utilizing induction chemotherapy as an individualized bioselection between CRT and TL as one of the treatment alternatives. A single cycle of cisplatin and 5-fluorouracil was administered to these patients followed by clinical treatment response assessment. Patients with tumors responding to induction therapy further underwent CRT, whereas patients with non-responsive tumors underwent TL. DSS for this bioselection group was superior to the CRT group and similar to the TL group. This approach may prove useful in the pursuit of better outcomes for organ-preservation therapy in LSCC, although more studies are warranted. Recently, new surgical techniques, namely transoral robotic surgery, have been introduced for organ-sparing LSCC management, especially in the case of supraglottic LSCC, with encouraging results (206,207).

6.2.2 RECURRENCE (STUDY II)

Despite successful primary treatment, LSCC has a tendency to recur. Recurrence has been reported to occur in up to 30% of cases (102-104). Variability in this tendency among different tumor stage groups is evident (11). In the current study, only 11% of T1 LSCC tumors recurred whereas recurrence was more common (21-39%) in T2-3 tumors. Other groups have reported similarly low recurrence rates for T1 LSCCs (106,208).

Currently, the European Laryngological Society recommends a 5-year post-treatment follow-up in LSCC (86). According to numerous studies, the majority of recurrences occur within three years of treatment completion and recurrences are rare thereafter (11,102,105,209,210). In the current cohort, the median time to recurrence was 9 months, with only 6 recurrences presenting after 36 months of follow-up. Somewhat later recurrences were observed by Canis et al. (106), who reported T1a glottic LSCC recurrences to occur as late as 131 months after treatment (median time to recurrence 34 months).

The advantage of the full 5-year follow-up over shorter follow-up for head and neck SCC remains unproven. Pagh et al. (211,212) conducted two cross-sectional studies regarding follow-up activities for head and neck SCC patients in Denmark. They observed a scarcity of late treatment-related morbidity and recurrences, proposing a limitation of routine follow-up to 3.5 years after treatment. They also observed an incidence of asymptomatic recurrence of 1 in 99 surveillance visits. Agrawal et al. (213) also examined the role of symptoms in the detection of recurrences and

observed that 79% of the patients diagnosed with recurrences were symptomatic. This rate was even higher (85%) in another study by the same group (214). However, 73% of these symptomatic recurrences were diagnosed at routine surveillance visits rather than at an earlier time point. Thus, anticipation of an upcoming surveillance visit may even delay the diagnosis of recurrence.

The utility of surveillance from the oncological point of view is the timely detection of treatable recurrences. Matoscevic et al. (215) observed a significantly reduced salvage rate for patients having locoregional versus local recurrences of LSCC. Agrawal et al. (214) observed significantly better post-recurrence survival for patients with primary early stage disease and isolated local recurrence. High salvage rates have also been reported for recurrent early LSCCs by TLS (16). The aforementioned results may imply that, in fact, the low risk patients may benefit more from follow-up than high-risk patients. High-risk patients, however, frequently have more treatment-related morbidity and need for physiotherapy, speech therapy services, and psychosocial support, thus justifying their close follow-up during the first post-treatment years.

A WHO performance status over 0 was found to be an independent predictor of recurrence in general and also a predictor of local recurrence in the current study. Cuny et al. (7) reported lower RFS for patients with WHO performance status >1. These findings were shared by Smee et al. (8), who observed higher incidence of local failures in patients “unfit for surgery”, although this factor was only significant in univariate analysis. However, when the endpoint was set to ultimate local failure, this factor also proved to be an independent prognostic factor. The general health status of the patient seems to have an independent impact on disease control, irrespective of tumor stage or treatment. Studies supporting the observation of female gender as a predictor of regional recurrence could not be found.

Non-glottic (mainly supraglottic) tumor location was also found to be an independent factor predicting regional recurrence in the current study. Johansen et al. (11) and Brandstorp-Boesen et al. (89) observed similar findings on the impact of tumor localization on recurrence (10). The presence of primary nodal metastasis as a general predictor of recurrence is supported by several studies, although the observation of this presence being an independent predictor of distant metastasis was not made in the current study, possibly due to the relatively small number of patients in this subgroup (93-95).

The impact of treatment choice on outcome has been one of the main topics of LSCC management. While the treatment outcomes in the USA are declining (4) and only slightly improving in Scandinavia (5), serious concerns regarding the feasibility of organ-sparing therapy have been raised (75-77). In the current retrospective series, primary non-surgical treatment was identified as an independent predictive factor for recurrence in general and also for local recurrence. A study based on the Netherlands Cancer Institute material revealed a significantly lower recurrence rate for T3-4 LSCC patients undergoing TL (13%) compared with those who received RT (32%) or CRT

(30%) (66). Although most of the studies addressing this issue are retrospective, it is logical to expect better LC after TL. TL may be considered a form of wide-field surgery, or even "compartmentectomy" of the entire laryngeal cancer site where dysplastic mucosa may be left behind after larynx-sparing treatment. Despite these results, there seems to be no difference in survival between the treatment groups.

When LSCC recurs, the most common mode of recurrence is an isolated local recurrence, which comprises over 50% of all recurrences (107,110). In this study, 68% of the recurrences were in the primary tumor site only. Local recurrences can often be successfully salvaged with good prognosis after treatment (118,216-220). Concurrent with these findings, a good 5-year post-recurrence DSS of 74% for patients with isolated local recurrences was observed in the current study. In contrast, more widespread recurrences translated to poor prognosis. Matoscevic et al. (215) also reported a drop in 5-year OS from 70% to 20% when locoregional recurrence instead of local recurrence only was detected. Brenner et al. (107) reported 2-year survival figures of 75% for local, 57% for regional, and 33% for distant recurrences. None of the patients with non-glottic tumor recurrence could be permanently salvaged in the current material. Sessions et al. (93) noted a similar, poor prognosis for supraglottic SCC recurrence, a 5-year DSS of only 17%. These results underline the importance of adequately aggressive primary treatment especially in the supraglottic patient group.

6.3 SURVIVIN, WRAP53β, AND P16^{INK4A} IN LSCC (STUDY III)

Currently, no reliable, clinically applicable means exist to stratify LSCC according to radiosensitivity. Therefore, it is important to identify an easy to assess, reproducible marker (or a panel of markers) with high sensitivity and specificity to enable the tailoring of treatment according to radiosensitivity and to achieve better outcomes for radioresistant tumors.

In previous studies, strong survivin expression has been associated with improved treatment outcome in a group of patients with various types of head and neck SCC (160,221). In the current study, with more homogenous patient material of T2-3N0 glottic LSCC treated with RT or CRT, the association was weaker. Only a trend towards better DFS was observed when the patients with positive nuclear expression were analyzed separately. A trend for better DFS in patients with strong nuclear expression was observed. Survivin expression was more heterogeneous in the current study compared to the data by Farnebo et al. (160), where the expression was predominantly nuclear. LSCC may present a different survivin expression pattern compared to head and neck SCC in general. Marioni et al. (222) studied the role of survivin in patients operated for LSCC. In their study, 83 patients with variable LSCCs of different T and N classes were assessed for tumor survivin expression. Strong nuclear survivin expression was associated with an increased rate of recurrence and decreased DFS. They also examined a panel of biomarkers, including

survivin, for predicting LSCC recurrence in patients undergoing post-operative RT. In their study, nuclear survivin expression was associated with poorer outcomes as well (223). This negative predictive role of survivin was also observed by Lo Muzio et al. in a series of oral cavity SCC patients (224). Different cellular localization and subtypes of survivin may explain these contradictory results (225). These contradictory results may also reflect the different treatment approaches across these studies.

In the current study, a trend towards poorer OS and significantly poorer DFS was observed for patients whose tumors revealed positive cytoplasmic staining for Wrap53β compared to those staining positive in the nucleus or those with negative staining. Silwal-Pandit et al. observed a similar poor outcome for breast cancer patients with tumors staining positive for Wrap53β in the cytoplasm but negative in the nucleus (165). Zhang et al. (226) examined Wrap53β expression in rectal cancer patients who were either treated with preoperative RT and surgery or surgery alone. Wrap53β downregulation was observed after preoperative RT. In the RT group, Wrap53β expression in the primary tumor had no effect on patient survival. However, expression of Wrap53β in metastases improved RT outcome. Unfortunately, information on the subcellular localization of Wrap53β was not available for comparison. The authors proposed that Wrap53β may be associated with the apoptotic pathway and Wrap53β downregulation may eventually lead to cell death. As the known functions of Wrap53β take place in the nucleus, it has been suggested that trapping of Wrap53β in the cytoplasm may hinder these functions, for example repair of DNA double-strand breaks (166,227). Wrap53β downregulation in the nucleus may also lead to telomere dysfunction, increasing radioresistance (228,229). These findings support our observation of cytoplasmic Wrap53β as a negative prognostic marker.

In oropharyngeal SCC, $p16^{INK4a}$ positivity has recently been recognized as a marker for improved treatment outcome (168,172). This improvement seems to be independent of treatment choice (230). However, in other head and neck sites, no such clear role for this marker has been shown to date (15,174,175,177). In the current study, $p16^{INK4a}$ positivity was rare and was associated with younger age (<60 years). In this younger population, no recurrences were detected in patients with $p16^{INK4a}$-positive tumors. Similar trends have been reported by some studies (174,175). Although not a significant marker in the general population of LSCC, $p16^{INK4a}$ may play a role in the pathogenesis and disease course in the subgroup of younger patients. While non-smokers had a higher prevalence of $p16^{INK4a}$ positivity in the current material, the number of these patients was too small for outcome comparisons with smokers.

Contrary to expectations, no associations between the above-mentioned markers and the presence of residual tumor after primary treatment could be detected. This may be due to the small number of patients having residual tumors after primary treatment (9%), which inevitably makes comparisons between protein expression and the initial success of therapy statistically insignificant if the association is not very

strong. Later recurrences, however, may be due to the proliferation of surviving radioresistant tumor cell populations. Accordingly, the assessment of especially DSS, DFS, and RFS may be reasonably considered to act as surrogate endpoints for radiosensitivity assessment. However, there may yet be some unknown mechanisms associated with these markers besides alterations in radiosensitivity that affect these outcomes.

6.4 BNCT IN THE TREATMENT OF RECURRENT LSCC (STUDY IV)

The current study is the first published series examining BNCT in the treatment of persistent or recurrent cancer of a single head and neck site with uniform histology, namely LSCC. Only two patients were found with LSCC from a previous series regarding BNCT in head and neck cancer; no information regarding their individual treatment outcomes is available.

The observation of overall response rate (75%) of persistent or recurrent LSCC to BNCT in this study is consistent with previous series regarding BNCT for head and neck cancer of variable sites and histologies (138,139,141,146,231). Despite initial positive treatment responses, durable disease control was achieved in only one patient with a small (1.4 cm in diameter) recurrent tumor. Several possible explanations for this may be speculated. Firstly, although a small sample size study by Wittig et al. exists on BPA-F uptake in head and neck SCC tissue (131), no data exists on BPA-F absorption to LSCC tissue in particular. Uptake of BPA-F by LSCC tissue may be different from other head and neck tumors, possibly leading to inadequate radiation dose. Secondly, dose calculations are difficult due to the lack of validated, accurate, and quantitative means to directly monitor BPA-F concentration in the tumor tissue itself. This may lead to under- or over-estimations of the delivered radiation dose, which can lead to increased toxicity or inadequate dose. Since tumor tissue is hard to obtain and analyze during the treatment process, other means of dose estimation are being sought. ^{18}F-BPA-PET is often conducted to verify tumor loading with BPA-F. However, no knowledge exists on the relation of findings on PET-CT scans to the actual concentration of BPA-F in tumor tissue, although PET-CT often demonstrates the dualistic accumulation of BPA-F to the tumor tissue while the concentration in normal tissues is low (139,232,233).

BNCT for recurrent LSCC was associated with a favorable toxicity profile. No Grade 4-5 early or late toxicity was observed and overall, acceptable rates of toxicity were encountered. This finding is in agreement with previous studies on BNCT in head and neck cancer, although other authors have reported some serious adverse effects. For instance, Wang et al. (146) and Suzuki et al. (138) reported incidences of carotid blowout after BNCT for head and neck carcinoma in 1 out of 17 and 3 out of 62 patients, respectively. Only one of these patients survived. Ideally, BNCT targets tumor cells sparing the surrounding healthy tissues from increased toxicity associated

with high radiation doses. Adverse effects may partly be sequelae of the aforementioned inaccurate dose estimations that are currently in use. When comparing BNCT to conventional reirradiation, toxicity is similar or lower (123,125,126).

6.5 FUTURE PERSPECTIVES

LSCC incorporates a wide spectrum of tumors with varying biological behavior and treatment strategies. As demonstrated, different treatment approaches may produce comparable treatment outcomes for similar TNM class tumors in selected patient materials. However, in every subsite and TNM category, excluding T1a glottic LSCC, recurrences are common and seriously compromise the goal of a cancer-free, high quality of life. With regard to the results of the current study, prospective studies on the role of prognostic markers for different treatment approaches (especially non-surgical treatment) should be conducted on sufficiently homogenous patient materials to obtain tools to counteract the observed subpar survival outcomes in certain patient groups, e.g. T2 tumors in the current study. Risk-group stratification (enabling treatment intensification for high-risk patients) and treatment de-intensification (to avoid adverse events where possible) would also improve the quality of LSCC treatment. Furthermore, follow-up protocols could be tailored to meet the individual patient's risk profile and needs, aiding in directing resources more efficiently. This risk-stratification may not necessarily be achieved by solitary markers alone (e.g. Wrap53β), but rather with panels of clinical and molecular markers. Large-scale, multi-center, and even international studies are needed to achieve sufficient numbers of patients for distinct patient subgroups.

Despite future advances in risk-stratification and treatment tailoring, recurrences will inevitably continue to present a management challenge in LSCC. TL produces favorable outcome but at the expense of laryngeal function. New technological advances in laryngeal surgery, e.g. transoral robotic surgery and new functional reconstruction techniques, may improve the oncological and functional outcomes of organ-sparing laryngeal surgery. New, more targeted forms of RT, e.g. proton therapy and BNCT, are being brought into investigational and clinical practice. In BNCT, the need for further refinement of the treatment protocol, i.e. more precise dose estimation and treatment tailoring, is evident. To achieve this, dynamic boron concentration development studies on tumor tissue are needed. Distinct studies should be conducted for different tumor sites and histologies, as the development of boron concentration in various localizations and histologies may vary. Also, more selective carriers for boron other than BPA-F or BSH are needed to optimize the tumor-to-tissue concentration ratio to further reduce treatment toxicity and thus enable dose escalation.

One of the advantages of BNCT is the ability to deliver high doses of RT over only 1-2 sessions compared to the conventional RT protocols. As different aspects of BNCT are

being explored, the spectrum of BNCT indications may also broaden with time. BNCT has already been investigated as a part of the primary oncological treatment (144). Although no reports exist so far, BNCT may also be experimented with in the future in the post-operative, adjuvant setting for recurrent tumors. With the development of clinic-based cyclotrons, epithermal neutrons are becoming increasingly available for clinical use in the future, compared to the conventional nuclear reactor source. Currently, a BNCT center utilizing this technology is being constructed in Helsinki. With this development, large-volume, prospective studies are expected to follow.

7 CONCLUSIONS

1. The outcome for T1a glottic LSCC in Finland is excellent. However, the outcome of patients with T2 glottic and supraglottic tumors was poorer than expected. CRT is increasingly used in the treatment of T3-4 LSCC. For T3 glottic LSCC, CRT produces better outcomes than RT. Based on the experiences from study I, CRT cannot be recommended for glottic T4 patients.

2. LSCC recurrences are rare after 36 months of follow-up, questioning the need for the standard 5-year follow-up for all LSCC patients, especially those with T1a glottic LSCC. WHO performance status >0, regional metastasis at presentation, and non-surgical primary treatment are independent predictors of recurrence. Local recurrences after glottic LSCC carry a good chance for cure. All other types of recurrences are associated with poor outcome.

3. Predominantly cytoplasmic Wrap53β expression is associated with significantly poorer DFS and a trend for poorer OS in T2-T3N0 glottic LSCC patients treated with non-surgical therapy. $p16^{INK4a}$ positivity is more abundant in younger patients, indicating a possible etiological role for HPV and a reduced risk of recurrence in this subgroup.

4. BNCT is safe in the treatment of LSCC recurrence. Although initial tumor response to BNCT is common, successful salvage of recurrent LSCC with BNCT alone according to the current protocol is rare.

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10 ORIGINAL PUBLICATIONS